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Development of the Skeletal Tissues and Skeletal Muscles in the Red Sea Bream

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ABSTRACT

The developmental process of the skeletal tissues and skeletal muscles in the red sea bream, *Pagrus major*, was revealed in relation to swimming and feeding functions. In addition, some morphological comparisons were made to clarify the differences between wild and reared specimens.

The developmental process of all osteological elements was described in detail. Larvae at first-feeding stage exhibited only some elements of the head skeleton and pectoral fin-supports. The completion of osteological structures was characterized by the formation of accessory cartilages 7 and 8 of the caudal skeleton and the ossification of the middle radials of the dorsal and anal fin-supports. An attempt to summarize the osteological development was made on the basis of the counts of considerable changes for respective elements. The cumulative percentage of the counts of progressed changes seemed to show the "developmental degree of osteological characters". In the head skeleton except the neurocranium, this value at first-feeding stage was considerably higher than other skeletal groups and rapidly increased later. The early formation of this skeletal group seemed to be concerned with its fundamental importance for larval survival. A histogram of the counts of considerable changes against total length consisted of several peaks and suggests the ontogenetic intervals in this species.

Histochemical characters of the fiber types of the myotomal muscle were confirmed and their developmental process was investigated. Three main fiber types, the so-called red, pink and white, were recognized. Newly hatched larvae were provided with a single layer of presumptive myoblasts of the superficial fibers surrounding the inner white fiber. The metabolic features referable to the red fiber type appeared in the superficial cells prior to first-feeding stage. It seemed that the superficial cells are able to serve initial sustained swimming activity at this stage. At about 7 mm TL, the pink fibers started to differentiate and the stratification of the red fibers occurred. This stage almost corresponded to that of increased swimming activity. It seemed that the stratification of the red fibers enables the larvae to begin true sustained slow swimming. The myotomal muscle almost reached adult condition in structure and function in early juveniles.

A morphometric examination of the development of the myotomal muscle fibers

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was carried out by observations of histological sections. Plots of the total number, the total cross-sectioned area and the average fiber area of the white and red fibers against total length on log/log co-ordinates were divided into three stanzas delimited by two inflections, respectively. In the case of the pink fibers, plots of their morphometric measurements were divided into two stanzas delimited by one inflection. Almost all first inflections of the white and red fibers occurred around first-feeding stage. All second inflections of the white and red fibers and the inflections of the pink fibers occurred between about 6 and 10 mm TL. It was suggested that the quantitative changes of the myotomal muscle follow the qualitative changes clarified by histological and histochemical observations and are correlated with changes in swimming activity.

The developmental process of the peripheral head muscles and fin muscles was described, using the graphical reconstruction technique of serial histological sections. The peripheral head muscles were first observed in a 2.95 mm TL larva, shortly prior to onset of feeding. The adductor mandibulae was formed as slender rod-like muscles. The levator arcus palatini was prominently large, compared with other muscles. It was assumed that this muscle may function for swallowing prey organisms, to supplement a thin adductor mandibulae in early stages. The conspicuous changes in the peripheral head muscles, such as the complete separation of the adductor mandibulae and formation of the tendon of A1, were recognized in 5.20 and 6.80 mm TL specimens. The differentiation of the peripheral head muscles was almost completed by 11.3 mm TL. The biting function seemed to appear through this process. The pectoral fin muscles in early stages were two flat muscles which serve an important role for propulsion. It was suggested that the pectoral fin muscles in later stages function for maneuverability rather than for propulsive swimming through the differentiation into eight muscle components. The caudal fin muscles also seemed to cause the complicated movement of the caudal fin as well as increase the propulsive force of tail beats in later stage.

The relationship between size and development of wild and reared specimens up to about 15 mm TL was examined. The numbers of the centra and rays of all fins, as characters representing the developmental levels, were counted and plotted against total length. The results apparently indicated that the development of the centrum and fin rays in reared specimens proceeds at smaller sizes than in wild ones. Possible causes of such size differences between wild and reared specimens in early stages were discussed. Contrary to the early development of the centrum and fin rays, it was suggested that the completion of osteological structures was accomplished at smaller sizes in wild specimens that in reared ones.

The meristic variation, bone abnormality and arrangement of vertebra-pterygiophore of juveniles were examined in wild and reared groups. Reared groups had considerably more variations than wild groups in meristic counts, such as the numbers of the vertebrae, pleural and dorsal ribs and fin rays. The detailed examination of bones revealed that the frequency of specimens with abnormal bones in reared groups is at least 5 times higher than in wild groups. The number of abnormal bones per individual in reared groups was at least 10 times higher. Specimens with either abnormal bone or abnormal arrangement of vertebra-pterygiophore amounted to 94% and 100% in reared groups, contrary to 26.0% and 28.8% in wild groups. It was suggested that rearing conditions may intensely influence abnormal bone formation.

Some considerations were made on the ontogenetic intervals, swimming and feeding functions and problems in seedling of this species. BALON's modified proposals on the

ontogenetic intervals were adopted to those of this species, consisted of periods and phases. The saltatory processes of qualitative changes were suggested from one period to another, and from one phase to another. The development of swimming and feeding functions seemed to be most important for larval survival through successful food uptake, avoidance from predators and migration. The possibility to produce healthy and vigorous fish was discussed. It was suggested that incidence of slight bone abnormality and abnormal arrangement of vertebra-pterygiophore may be useful as an indicator for qualitative evaluation of reared juveniles.

CONTENTS

INTRODUCTION

The red sea bream, *Pagrus major* (TEMMINCK et SCHLEGEL), is one of the most important commercial fish in Japan. To satisfy an increasing demand for this species, farming is now extensively performed, using wild-caught larvae or artificially-reared larvae. Recently, vigorous efforts for stocking recruits of this species are made in many parts of Japan by releasing artificially-reared juveniles along coastal areas.

The rapid advance of marine fish rearing technique for the last two decades is greatly due to two main reasons. The first reason is the development of mass-culture method (including nutritional improvement) of the rotifer, *Brachionus plicatilis*, as larval feed, and second is the utilization of a large amount of viable eggs spawned naturally by parent fish in culture-pond instead of artificially-fertilized eggs. Numerous rearing and farming experiments of *P. major* have been made and their outlines are reviwed by YAMAGUCHI.[1] In quite recent years, mass production of seedling becomes possible and over ten million juveniles of this species are artificially produced. The majority of these juveniles are released along coastal areas for stocking recruits of this species.

In spite of these improvements in rearing technique, there has been relatively little knowledge concerning the larvae themselves, especially on developmental aspects of functional morphology, physiology, behaviour, etc. To expect further improvement in seedling and stocking recruits of this species, more biological information on the early life history should be accumulated, as pointed out by FUKUHARA.[2]

Investigations on the development of *P. major* have been mainly devoted to the gill,[3] swimbladder,[4,5] lateral line,[6,7] chloride cell,[8] eye,[9] digestive system,[10-12] bone,[13-15] squamation,[13,16] ray,[17,18] mouth size,[19,20] swimming behaviour,[21,22] etc. No information as to the development of the skeletal muscles of *P. major* has ever been presented. Likewise, there has been no detailed examination devoted to the developmental process of all skeletal elements, from their initial appearance to completion.

The present study was undertaken mainly to clarify the developmental process

of the skeletal and muscular structures of *P. major*. These structures are closely related to functions important for larval survival such as swimming, feeding, respiration, etc. Therefore, a detailed study of the developmental process of these structures will provide some basic knowledge for further improvement of production of larvae and, probably, ecological understanding of this species. Moreover, to produce healthy and vigorous larvae which can successfully survive and grow in the natural environment, a detailed comparative study between wild and reared stocks is needed. But there is little literature related to this problem.[18,23-25] In this study, examination of the osteological (including osteological development) differences between wild and reared larvae of this species was also undertaken. Clarifying such differences may contribute to eliminating their cause and subsequently producing reared larvae which will be more similar to its wild counterparts.

I. OSTEOLOGICAL DEVELOPMENT

The osteological development of *P. major* has been already partly described by some authors. Kitajima[13] indicated brief observations of the vertebrae and fins and fin-supports of larvae and juveniles. Takashima[14] also showed the development of the vertebrae, especially in relation to the formation of abnormal vertebrae. These observations, however, were made only for the bone of alizarin red S-stained specimens. Since Dingerkus and Uhler[26] developed the convenient double staining method for bone and cartilage, using alizarin red S and alcian blue, information on the cartilaginous-osteological development of teleostean fishes has been increasing (see, Kohno and Taki,[27] Potthoff *et al.*[28]). Kohno *et al.*,[15] using this technique, examined the osteological development of some skeletal parts in a relatively limited range of sizes for *P. major*. In this chapter, the detailed developmental process of all osteological elements of *P. major*, except the scale, was described from initial appearance to completion, and compared with the results reported by other authors. Some discussions were given in relation to the development of functional aspects of these organs.

Some parts of this chapter have already been presented by Matsuoka.[29,30]

Materials and methods

Series of 376 laboratory-produced specimens from prelarvae to juveniles up to 40.0 mm TL, reared in Fukui Prefectural Fisheries Farming Center in 1980, were used for most of the descriptions. Supplemental observations were made on 106 specimens (prelarvae to 72.0 mm TL) reared in Aquaculture Research Laboratory, Nagasaki Prefectural Institute of Fisheries in 1977, and two wild specimens (111.0

and 151.0 mm TL) caught in Wakasa Bay in 1980.

Specimens fixed in 10% formalin solution were cleared and stained, using the method of TAYLOR[31] for bone, and the method of DINGERKUS and UHLER[26] for both bone and cartilage.

Total length was measured after clearing and staining.

For histological observation, some specimens fixed in Bouin's solution were embedded in paraffin, sectioned at 5–8 μm, and stained with hematoxylin, eosin and alcian blue.

Bone terminology generally followed HOUDE and POTTHOFF,[32] LEIBY[33,34] and ROSEN and PARENTI.[35]

Results

Neurocranium

In a 2.90 mm TL specimen, shortly prior to onset of feeding, the ethmoid plate, trabecula and parachordal cartilages were present and joined together. The laterventral parts of the auditory capsules were chondrified and connected with the parachordals (Fig. 1A).

In a 4.35 mm TL specimen, the supraorbital bars and occipital processes were chondrified (Fig. 1B).

In a 5.90 mm TL specimen, the complete cartilaginous auditory capsules were formed. The epiphysial tectum and ectethmoid bars were chondrified. The frontals and parasphenoid were formed by fibrous ossification. The ethmoid cartilage was a small dorsal flange on the ethmoid plate cartilage. There was no direct cartilaginous connection between the trabecula and auditory capsule cartilage. This interspace was replaced by the parasphenoid (Fig. 1C).

The parietals (fibrous ossification), epiotics, pterotics, sphenotics, prootics, supraoccipital, exoccipital and basioccipital were almost simultaneously ossified at about 6.5 mm TL. The basioccipital was initially formed by fibrous ossification, surrounding the anterior tip of the notochord, but later the ossification extended to the adjacent cartilaginous region.

In a 8.50 mm TL specimen, the frontals fully extended and made up the dorsal wall of the neurocranium. The nasals, vomer and intercalars were formed by fibrous ossification. The parasphenoid extended along almost the entire length of the base of the neurocranium. A small cartilaginous basisphenoid existed on the parasphenoid. The ethmoid cartilage fully developed and the lateral ethmoids were ossified (Fig. 1D).

The pterosphenoids were preformed as cartilage and later ossified at about 9 mm TL. The ethmoid and basisphenoid were ossified at about 9 and 10 mm TL,

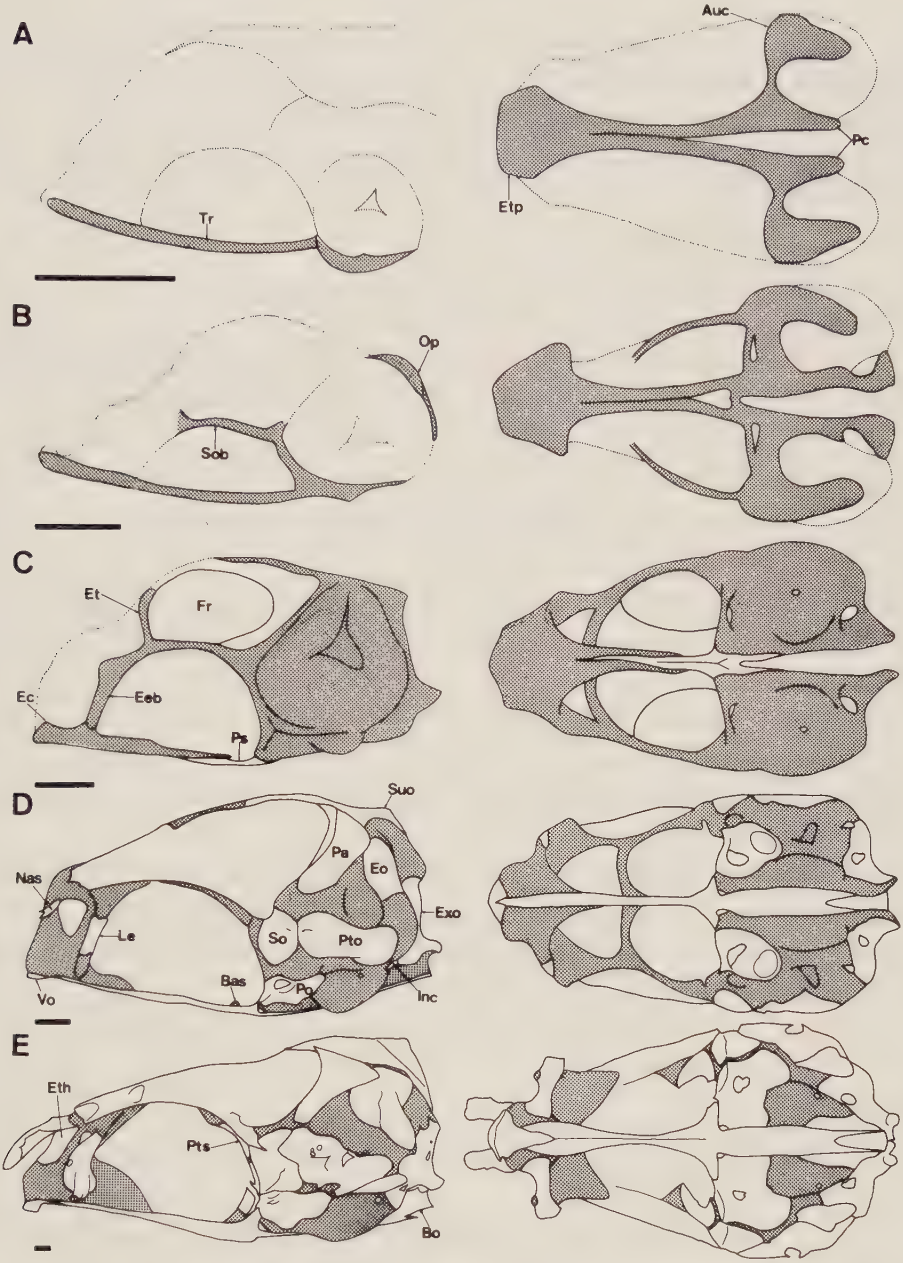


Fig. 1. Development of neurocranium in *Pagrus major*. A: 2.90 mm TL. B: 4.35 mm TL. C: 5.90 mm TL. D: 8.50 mm TL. E: 20.1 mm TL. Left, lateral view. Right, ventral view. Stippled area, cartilage. Open area, ossified portion. Auc, auditory capsule; Bas, basisphenoid; Bo, basioccipital; Ec, ethmoid cartilage; Eeb, ectethmoid bar; Eo, epiotic; Et, epiphysial tectum; Eth, ethmoid; Etp, ethmoid plate; Exo, exoocipital; Fr, frontal; Inc, intercalar; Le, lateral ethmoid; Nas, nasal; Op, occipital process; Pa, parietal; Pc, parachordal; Po, prootic; Ps, parasphenoid; Pto, pterotic; Pts, pterosphenoid; So, sphenotic; Sob, supraorbital bar; Suo, supraoccipital; Tr, trabecula; Vo, vomer. Scale bars indicate 0.2 mm.

respectively. All elements of the neurocranium appeared at about 10 mm TL, though the cartilaginous area still considerably existed in the neurocranium of a 20.1 mm TL specimen (Fig. 1E).

The intercalars became obscure in a 151.0 mm TL specimen, because of fusion with the pterotics.[36]

Sclerotic and infraorbital bones

In a 2.90 mm TL specimen, the lateral edge of the eye capsule was surrounded by the thin sclerotic cartilage or eye band (Fig. 2A).

In a 8.50 mm TL specimen, the lachrymal was formed by fibrous ossification (Fig. 2E). Five infraorbitals were also formed by fibrous ossification between about 9 and 13 mm TL.

The anterior ossified area in the sclerotic cartilage appeared at about 15.5 mm TL and the posterior one appeared at about 20 mm TL (Fig. 2F).

Jaws

In a 2.90 mm TL specimen, the Meckel's cartilage fully developed, framing the lower jaw, and the flagellumlike maxillary was formed on the upper jaw edge by weak fibrous ossification (Fig. 2A).

The dentary began to be formed outside the anterior region of the Meckel's cartilage by fibrous ossification by about 4 mm TL (Fig. 2B). The angular and retroarticular were ossified on the posterior region of the Meckel's cartilage by about 5 mm TL.

In a 5.90 mm TL specimen, all elements of the upper and lower jaws except the splenial appeared. The premaxillary (fibrous ossification) and rostral cartilage were present. Small premaxillary and dentary teeth also appeared (Fig. 2C), more visible in a 6.85 mm TL specimen (Fig. 2D).

The splenial (not shown in the figure) was ossified on the laterodorsal face of the posterior region of the Meckel's cartilage at about 8.5 mm TL.

Suspensorium

In a 2.90 mm TL specimen, the hyomandibular-symplectic and quadrate cartilage were present (Fig. 2A).

In a 4.35 mm TL specimen, the mid region of the palatine began to be chondrified (Fig. 2B).

By about 5 mm TL, the quadrate, symplectic and hyomandibular were ossified, and the cartilage contiguous to the quadrate extended to the metapterygoid region.

In a 5.90 mm TL specimen, the endopterygoid was formed by fibrous ossification (Fig. 2C).

In a 6.85 mm TL specimen, the ectopterygoid was formed by fibrous ossification and the palatine cartilage possessed the ossified ventral keel (Fig. 2D).

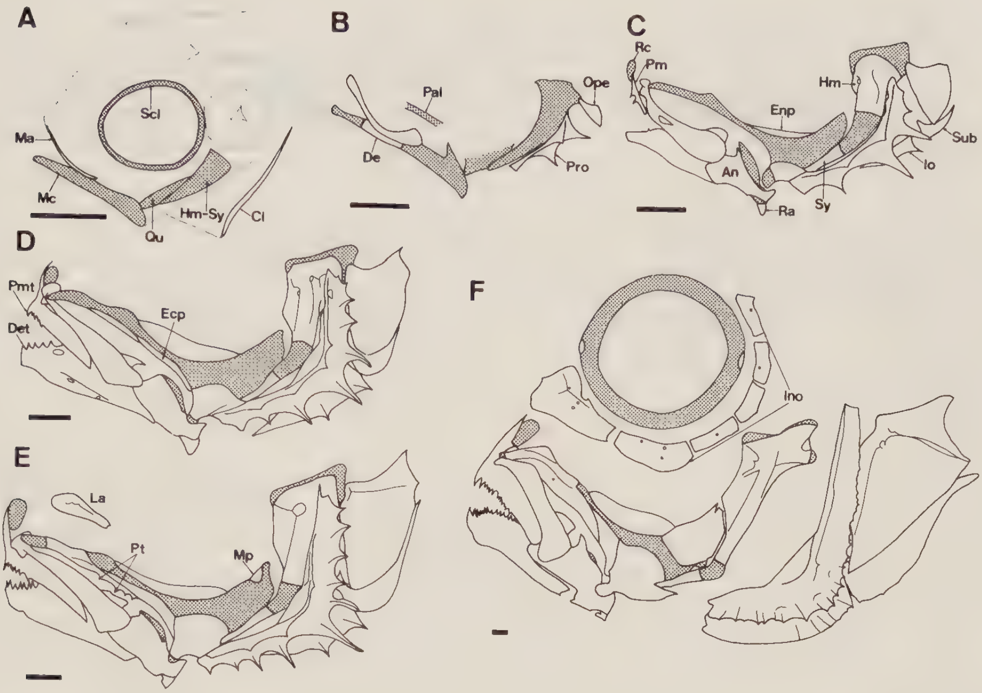


Fig. 2. Development of sclerotic and infraorbital bones, suspensorium, opercular bones and upper and lower jaws in *Pagrus major*. A : 2.90 mm TL. B : 4.35 mm TL. C : 5.90 mm TL. D : 6.85 mm TL. E : 8.50 mm TL. F : 20.1 mm TL. Lateral view. Sclerotic is removed in B-E. Stippled area, cartilage. Open area, ossified portion. An, angular ; Cl, cleithrum ; De, dentary ; Det, dentary teeth ; Ecp, ectopterygoid ; Enp, endopterygoid ; Hm, hyomandibular ; Hm-Sy, hyomandibular-sympletic cartilage ; Ino, infraorbital ; Io, interopercle ; La, lachrymal ; Ma, maxillary ; Mc, Meckel's cartilage ; Mp, metapterygoid ; Ope, opercle ; Pal, palatine ; Pm, premaxillary ; Pmt, premaxillary teeth ; Pro, preopercle ; Pt, palatine teeth ; Qu, quadrate ; Ra, retroarticular ; Rc, rostral cartilage ; Scl, sclerotic ; Sub, subopercle ; Sy, symplectic. Scale bars indicate 0.2 mm.

In a 8.50 mm TL specimen, the palatine cartilage was ossified in the mid region and a few small teeth were temporarily formed on the posterior ventral edge of the palatine. The metapterygoid was ossified (Fig. 2E).

The palatine teeth disappeared by about 15.5 mm TL (Fig. 2F).

Opercular bones

All elements of the opercular apparatus were formed by fibrous ossification. The preopercle and opercle appeared at about 3.5 mm TL (Fig. 2B). The interopercle and subopercle appeared at about 5 mm TL (Fig. 2C).

The preopercular and interopercular spines were noticeable in the earlier stages (Fig. 2B-E), but later became vestigial (Fig. 2F).

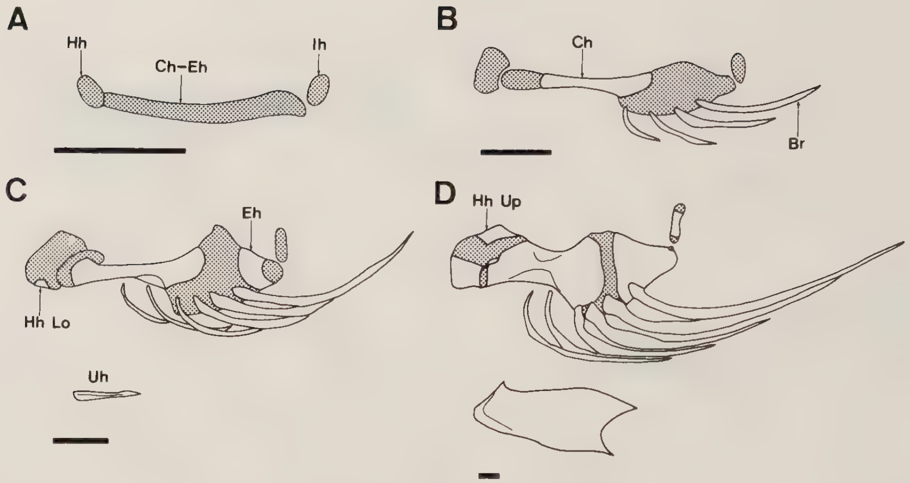


Fig. 3. Development of hyoid arch in *Pagrus major*. A: 2.90 mm TL. B: 5.90 mm TL. C: 6.85 mm TL. D: 20.1 mm TL. Lateral view. Basihyal is shown in Fig. 4. Stippled area, cartilage. Open area, ossified portion. Br, branchiostegal ray; Ch, ceratohyal; Ch-Eh, ceratohyal-epihyal cartilage; Eh, epihyal; Hh, hypohyal; Hh Lo, hypohyal lower; Hh Up, hypohyal upper; Ih, interhyal; Uh, urohyal. Scale bars indicate 0.2 mm.

Hyoid arch

In a 2.90 mm TL specimen, the hypohyal, ceratohyal-epihyal and interhyal cartilages were present (Fig. 3A).

In a 5.90 mm TL specimen, the ceratohyal was ossified in the anterior region of the ceratohyal-epihyal cartilage and four branchiostegal rays were formed by fibrous ossification (Fig. 3B). The basihyal was chondrified (Fig. 4C).

The branchiostegal rays began to be formed from posterior to anterior one at about 4.5 mm TL and the full complement of six rays was accomplished by about 6.5 mm TL.

In a 6.85 mm TL specimen, the lower hypohyal was ossified in the lower region of the hypohyal cartilage and the epihyal also ossified in the posterior region of the ceratohyal-epihyal cartilage. The urohyal was formed as a thin rod by fibrous ossification (Fig. 3C). The posterior tip of the basihyal was ossified (Fig. 4D).

The upper hypohyal was ossified at about 8 mm TL in the upper region of the hypohyal cartilage.

The urohyal gradually extended vertically with growth (Fig. 3D).

Branchial arches

In a 2.90 mm TL specimen, the basibranchial, two pairs (first and second) of the hypobranchials and four pairs (first to fourth) of the ceratobranchials were present as cartilage, but there was no trace of the upper branchial arches (Fig. 4A).

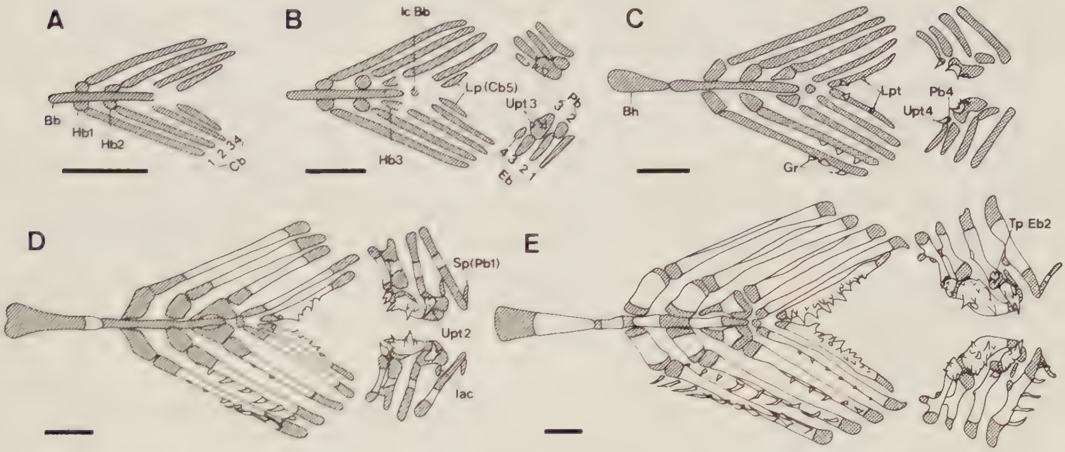


Fig. 4. Development of upper and lower branchial arches in *Pagrus major*. A: 2.90 mm TL. B: 4.35 mm TL. C: 5.90 mm TL. D: 6.85 mm TL. E: 10.0 mm TL. Upper branchial arches, ventral view. Lower branchial arches, dorsal view. Gill rakers of right body side are removed in C-E. Stippled area, cartilage. Open area, ossified portion. Bb, basibranchial; Bh, basihyal; Cb, ceratobranchial; Eb, epibranchial; Gr, gill raker; Hb, hypobranchial; Iac, interarcual cartilage; Ic Bb, isolated cartilage of basibranchial; Lp, lower pharyngeal; Lpt, lower pharyngeal teeth; Pb, pharyngobranchial; Sp, suspensory pharyngeal; Tp Eb 2, tooth plate of epibranchial 2; Upt, upper pharyngeal teeth. Scale bars indicate 0.2 mm.

The isolated cartilage of the basibranchial, third pair of the hypobranchials, a pair of the lower pharyngeals (fifth pair of the ceratobranchials), three pairs (first to third) of the epibranchials and two pairs (second and third) of the pharyngobranchials were chondrified between about 3.5 and 4 mm TL. A few upper pharyngeal teeth were also formed on the third pair of the pharyngobranchials.

In a 4.35 mm TL specimen, the fourth pair of the epibranchials was chondrified (Fig. 4B).

A few lower pharyngeal teeth appeared at about 5 mm TL.

In a 5.90 mm TL specimen, the fourth pair of the pharyngobranchials was chondrified, with a respective single tooth. The fourth pair of the pharyngobranchials remained cartilaginous even in the largest specimen examined, 151.0 mm TL. The gill rakers were present on the first and second pairs of the ceratobranchials (Fig. 4C).

Beyond about 6 mm TL, the four pairs of the ceratobranchials and a pair of the lower pharyngeals were ossified, and a pair of the suspensory pharyngeals (first pair of the pharyngobranchials) was chondrified.

In a 6.85 mm TL specimen, four pairs of the epibranchials and the second and third pairs of the pharyngobranchials were ossified. A few teeth were present on the second pair of the pharyngobranchials. A pair of rodlike cartilages, the inter-

arcual cartilage of ALLIS,[37] was present between the first epibranchial and second pharyngobranchial. The gill rakers began to be formed on the third pair of the ceratobranchials (Fig. 4D.)

The gill rakers on the fourth pair of the ceratobranchials were present beyond 7 mm TL. Three ossified regions of the basibranchial and the tooth plates on the second pair of the epibranchials were formed at about 7.5 and 8 mm TL, respectively. Three pairs of the hypobranchials were ossified between about 8 and 8.5 mm TL. A pair of the suspensory pharyngeals was ossified at about 10 mm TL. All elements of the branchial arches appeared at this stage (Fig. 4E).

Vertebral column

In a 4.50 mm TL specimen, no neural and haemal arch was observed, although the hypural 1 was chondrified (Fig. 5A).

In a 4.85 mm TL specimen, the second and third neural arches appeared as cartilage. Each neural arch consisted of two rodlike cartilages formed on the right and left laterodorsal faces of the notochord (Fig. 5B).

In a 5.20 mm TL specimen, the first to ninth haemal arch cartilages appeared. Each haemal arch consisted of two small cartilages formed on the right and left lateroventral faces of the notochord. Among these cartilages, the fifth one was the longest (Fig. 5C).

In a 5.30 mm TL specimen, many neural arch cartilages appeared. The first one was formed on the notochord, and elongated dorsally as well as the second and third ones, while the fourth to 14th ones first appeared on the dorsal side of the spinal cord and elongated ventrally. In the case of the 15th to 18th cartilages, they were simultaneously formed on both the dorsal sides of the spinal cord and notochord, and later fused at the mid lateral side of the spinal cord. The 19th neural arch cartilage (and also the 20th to 23rd ones) was formed on the notochord and elongated dorsally. The formation of the haemal arch cartilages proceeded posteriorly, except the 13th one which appeared earlier than several of the anterior haemal arches. The parapophysis cartilages (each consisting of two small cartilages), like the haemal arches, were formed beneath the notochord and their formation proceeded anteriorly (Fig. 5D).

In a 5.35 mm TL specimen, four parapophysis cartilages were formed and clearly distinguishable from the haemal arch cartilages, because of their shorter length. Therefore, it was possible to distinguish between the abdominal and caudal vertebrae after this stage. In the fourth to 14th neural arches, when the cartilage reached about 2/3 of the height of the spinal cord, small cartilage was newly formed on the notochord, which later fused with the original cartilage. This process occurred earlier in the anterior and posterior neural arches, with the seventh one the

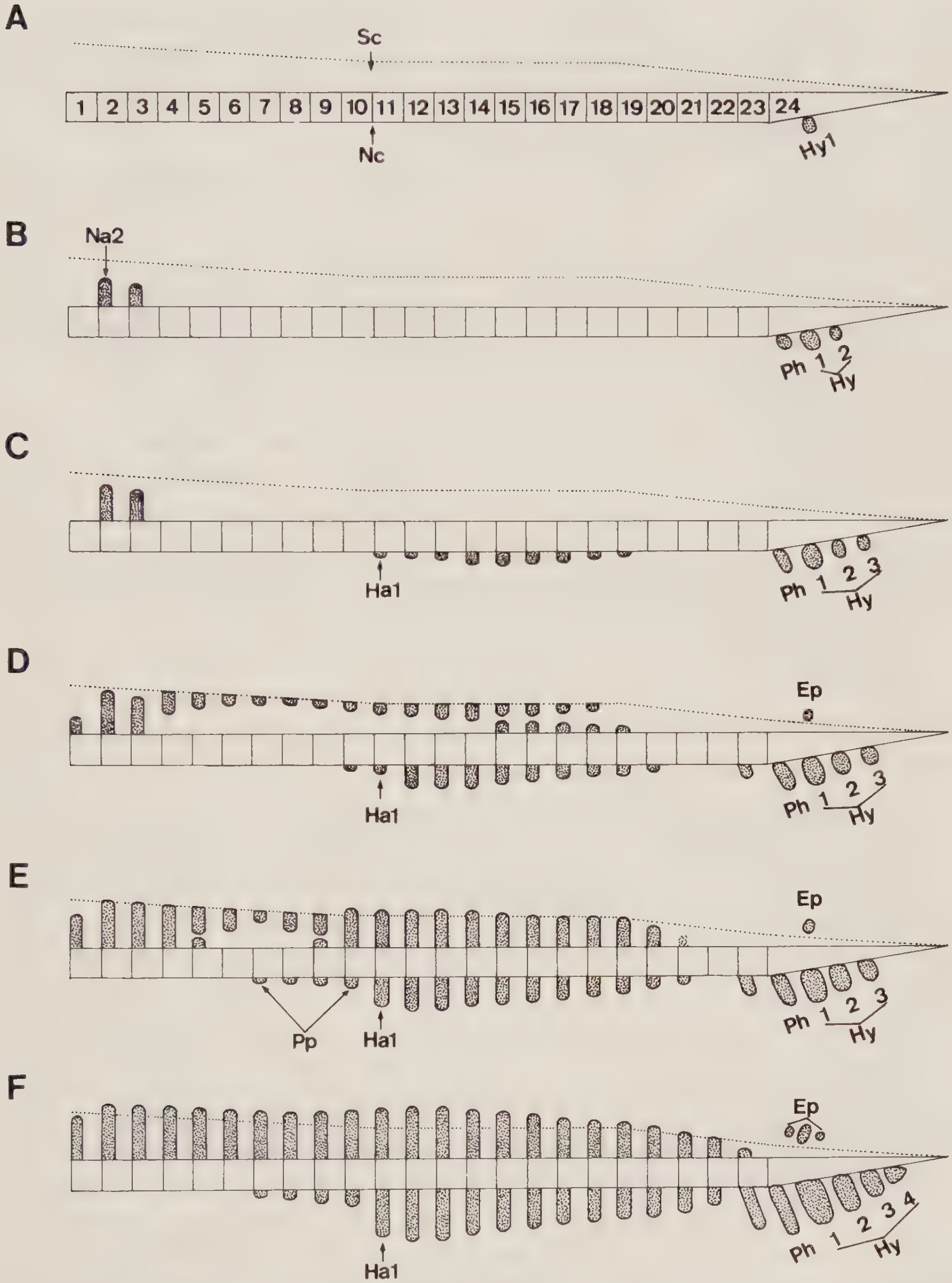


Fig. 5. Schematic representation of cartilage development of vertebral column and caudal skeleton in *Pagrus major*. A : 4.50 mm TL. B : 4.85 mm TL. C : 5.20 mm TL. D : 5.30 mm TL. E : 5.35 mm TL. F : 6.00 mm TL. Number in notochord region shows myomere number attached to notochord. Stippled area, cartilage. Ep, epural ; Ha, haemal arch ; Hy, hypural ; Na, neural arch ; Nc, notochord ; Ph, parhypural ; Pp, parapophysis ; Sc, spinal cord.

last to be completed. After the neural arch cartilage entirely surrounded the spinal cord, the neural spine cartilage began to elongate dorsally from the neural arch. The haemal spine cartilage also elongated ventrally from the haemal arch entirely surrounding the caudal artery and vein (Fig. 5E).

In a 6.00 mm TL specimen, all neural arch, haemal arch and parapophysis cartilages appeared, but no ossification in the vertebral column and formation of the centrum was recognized (Fig. 5F).

At about 6 mm TL, ossification began simultaneously with the dorsal flexing of the posterior end of the notochord. Ossification first occurred at the second neural arch and proceeded posteriorly. The formation of the centrum followed the ossification of the neural arch and also proceeded posteriorly (Fig. 6A). The ossification of the first neural arch and centrum occurred later than that of several posterior ones. The ossification of the parapophysis and haemal arch, like the neural arch, preceded the formation of the centrum in respective vertebra (Fig. 6 B). The urostyle (24th centrum) was ossified when the 16th or 17th centrum was formed (Fig. 6C). At about 9 mm TL, the 23rd centrum appeared and the full complement of the centra was accomplished (Fig. 6D).

Figure 7 represents the schematic developmental process of the 11th vertebra. The ossification centers of the neural and haemal arches were located at the mid lateral sides of the spinal cord and the caudal artery and vein, respectively. Ossification proceeded dorsally and ventrally from these centers. As the cartilage cells were replaced by the bony matrix, the ossified region became thinner (Fig. 7 A, B). When the ossification of the neural arch proceeded up to the dorsal side of the spinal cord, the centrum began to be formed on the notochord surface around the basal part of the neural arch. Simultaneously, the notochord around the centrum slightly sank and the prospective intervertebral regions were formed (Fig. 7C). The ossification of the centrum proceeded to the dorsal and lateral sides of the notochord and subsequently a "bony ring" of the centrum surrounding the notochord was formed (Fig. 7D). In the 22nd and 23rd vertebrae, however, the formation of the centrum first occurred around the basal part of the haemal arch and proceeded to the ventral and lateral sides of the notochord. When the basal halves of the neural and haemal spines was slightly ossified, their tips also began to be ossified, forming needlelike spines (Fig. 7D, E). The cylindrical centrum elongated posteriorly and consequently the intervertebral regions became narrow (Fig. 7E). The cartilaginous portions in each vertebra gradually disappeared. The centrum constricted at its center and showed the biconid amphicoelous shape. The posterior halves of the neural and haemal arches were formed by fibrous ossification, and surrounded the spinal cord and the caudal artery and vein, respectively (Fig. 7F–H).

Figure 8 shows a longitudinal section through the centrum in a 14.5 mm TL

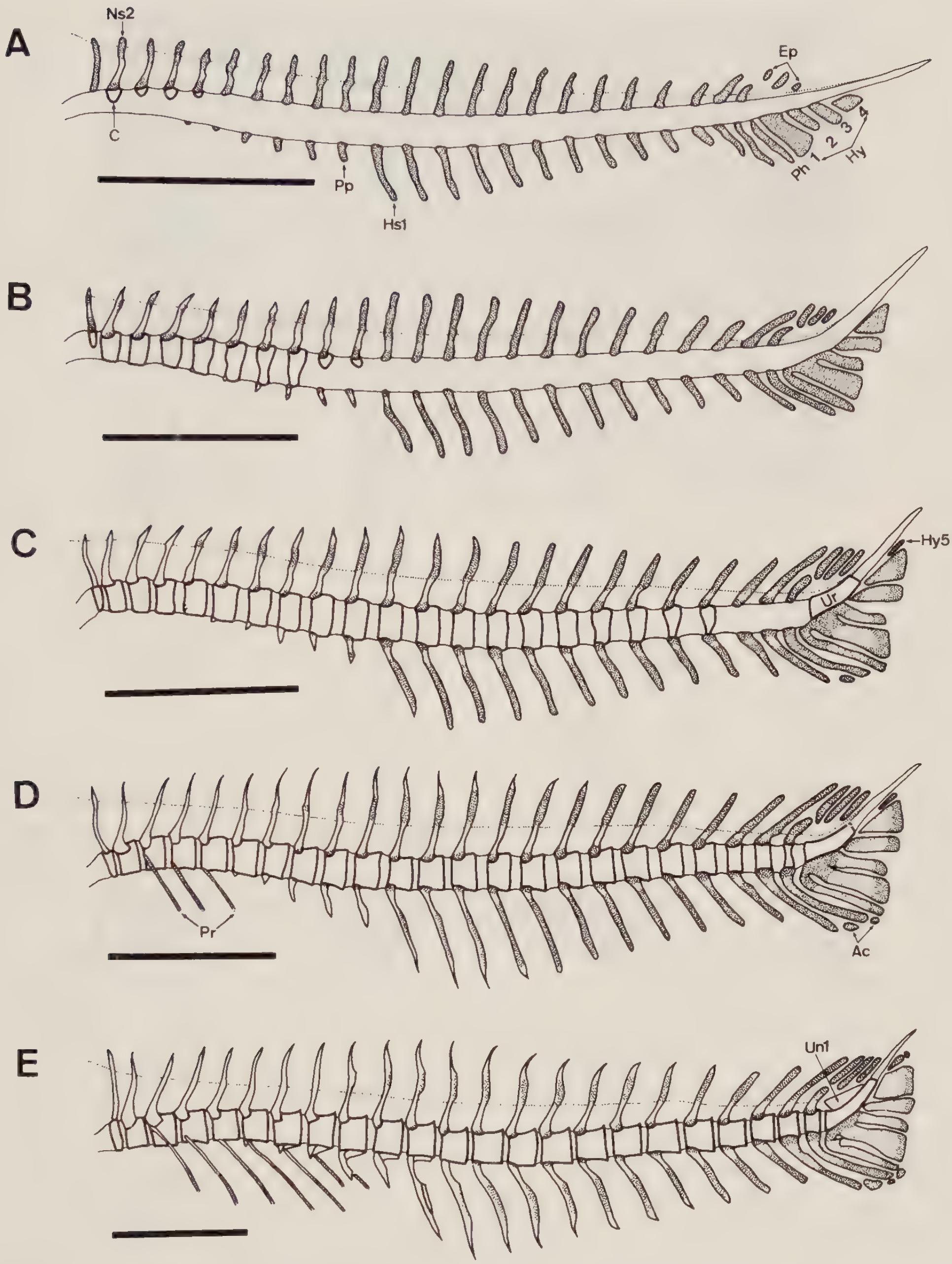


Fig. 6. Ossification sequence of vertebral column and caudal skeleton in *Pagrus major*. A: 5.85 mm TL. B: 7.05 mm TL. C: 7.25 mm TL. D: 8.80 mm TL. E: 11.8 mm TL. Stippled area, cartilage. Rough stippled area, weak ossification. Open area, developed ossification. Ac, accessory cartilage; C, centrum; Hs, haemal spine; Ns, neural spine; Pr, pleural rib; Un, uroneural; Ur, urostyle. For other abbreviations, see Fig. 5. Scale bars indicate 1 mm.

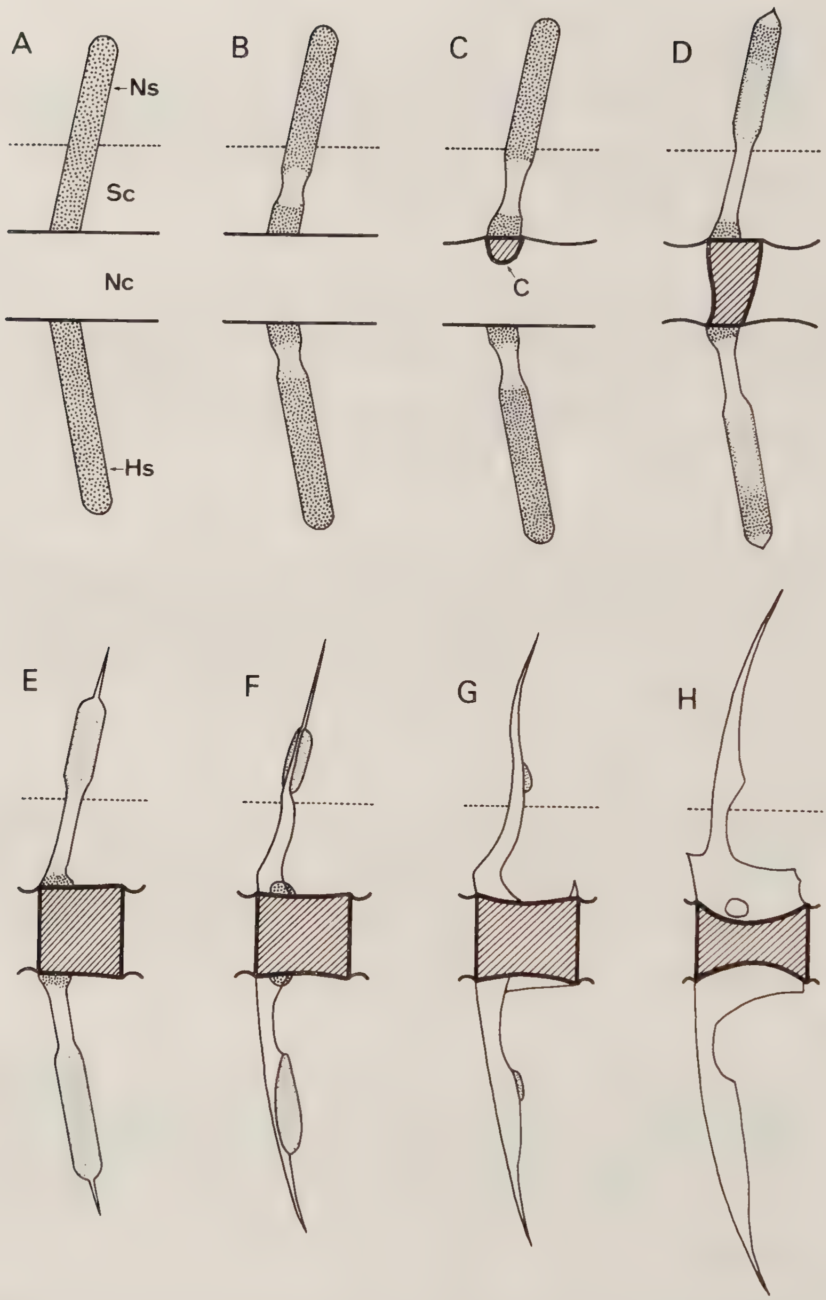


Fig. 7. Schematic representation of development of the 11th vertebra in *Pagrus major*. Stippled area, cartilage. Rough stippled area, weak ossification. Open area, developed ossification. For abbreviations, see Figs. 5 and 6.

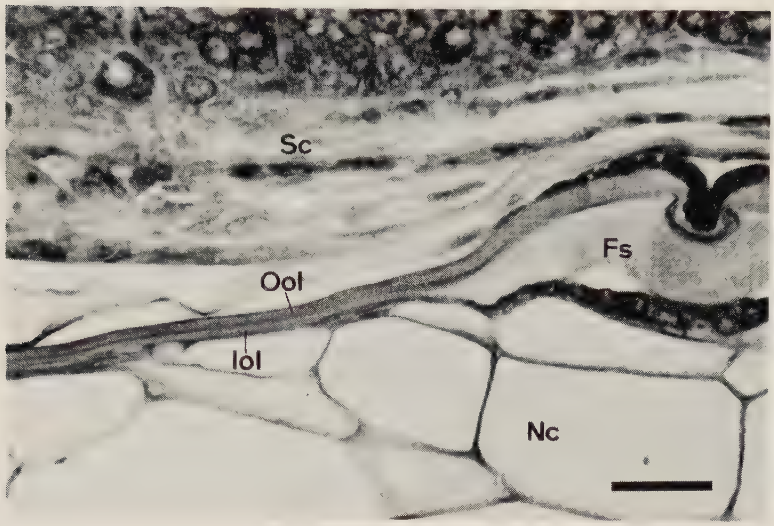


Fig. 8. Photomicrograph of histological section of centrum in *Pagrus major* (14.5 mm TL), showing double origin of ossification. Longitudinal section. Stained with alcian blue, hematoxylin and eosin. Fs, fibrous sheath; Iol, inner ossified layer; Nc, notochord; Ool, outer ossified layer; Sc, spinal cord. Scale bar indicates 20 μm.

specimen. The double ossified layers (outer and inner) of the centrum were recognizable. The thin inner layer was continuous with the thickening fibrous sheath in the intervertebral region. The thickening fibrous sheath was distinctly stained with alcian blue. On the other hand, the inner layer was slightly stained with eosin; it is an indication of decalcified osseous tissue.

The pleural and dorsal ribs are the appendages of the vertebral column. The former consisted of eight pairs corresponding to the third to tenth vertebra and the latter twelve pairs corresponding to the first to 12th one, although some meristic variations were recognized especially in reaed specimens.

The pleural ribs began to be formed as cartilage from anterior to posterior at about 8 mm TL. These cartilages first appeared between adjacent myomeres, near the abdominal cavity, somewhat apart from the vertebra, and elongated at both tips. The ossification occurred at about 8.5 mm TL and proceeded from anterior to posterior, although anterior three pairs seemed to be simultaneously ossified (Fig. 6D, E). The eighth pair was chondrified at about 18 mm TL, ossified at about 19 mm TL and fully ossified up to its ventral tip, forming a sharp spine, at about 21 mm TL.

The dorsal ribs began to be formed by fibrous ossification from anterior to posterior at about 9 mm TL. These first appeared along the boundary between the

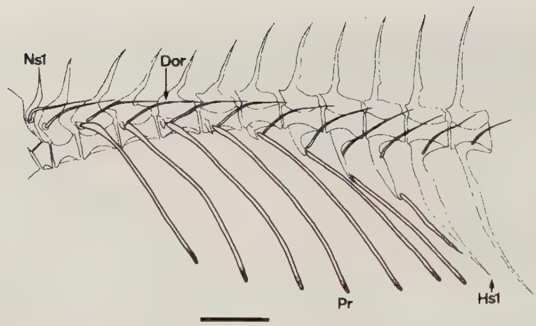


Fig. 9. Ventrolateral view of abdominal region of vertebral column with dorsal and pleural ribs in *Pagrus major*, 27.5 mm TL. Dor, dorsal rib. For other abbreviations, see Fig. 6. Scale bar indicates 1 mm.

myoseptum and horizontal septum, somewhat near the lateral side, and elongated at both tips. The full complement was accomplished by about 24 mm TL.

Figure 9 shows the ventrolateral view of the abdominal region of the vertebral column with the pleural and dorsal ribs of a 27.5 mm TL specimen. The first and second dorsal ribs were articulated with the lateral side of the neural arch, the third to eighth one with the base of the pleural rib, and the ninth to 12th one with the lateral side of the parapophysis or haemal arch.

Caudal fin-supports

The caudal skeleton of *P. major* consists of three centra (including the urostyle), one neural arch and spine, two haemal arches and spines, one parhypural, five hypurals, two pairs of the uroneurals, three epurals, one specialized neural arch, 17 (9+8) principal rays and eight accessory cartilages (or radial cartilages). To avoid overlapping, the developmental process of the elements, except the centrum, neural arch and spine and haemal arch and spine, are described here.

The hypural 1 was the first to be chondrified in the caudal fin-supports at about 4.5 mm TL (Fig. 5A). The parhypural and hypural 2-4 cartilages were successively formed by about 6 mm TL (Fig. 5B-F). Of the three epurals, the central one first appeared as cartilage in a 5.30 mm TL specimen, and the anterior and posterior ones were chondrified by about 6 mm TL (Fig. 5D-F). The hypural 5 cartilage was formed by about 7 mm TL (Fig. 10A). There was no cartilaginous junction among the parhypural, hypural 1 and 2 in early stages, but later the latter two elements were fused at the basal parts and subsequently joined with the former (Fig. 10A, B).

The rudiments of the principal rays began to be formed and ossified from central to peripheral at about 5.3 mm TL. The full complement was accomplished by about 7 mm TL.

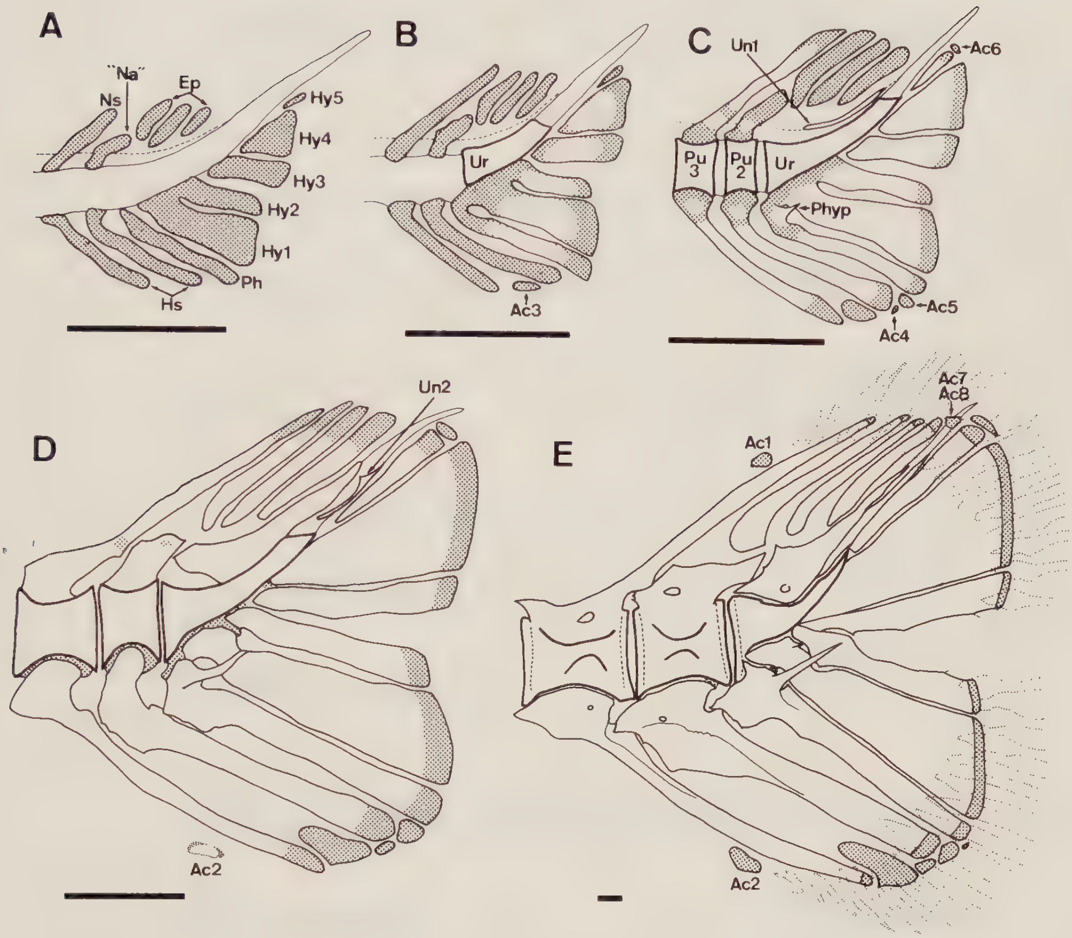


Fig. 10. Development of caudal skeleton and accessory cartilage in *Pagrus major*. A: 7.05 mm TL. B: 7.25 mm TL. C: 10.3 mm TL. D: 23.0 mm TL. E: 111.0 mm TL. Stippled area, cartilage. Rough stippled area, weak ossification. Open area, developed ossification. "Na", specialized neural arch; Phyp, parhypurapophysis; Pu, preural centrum. For other abbreviations, see Figs. 5 and 6. Scale bars indicate 0.5 mm.

The ossification of the parhypural and hypural 1–4 occurred at about 7 mm TL, preceding the urostyle formation (Fig. 10A, B). The hypural 5 was ossified at about 9 mm TL (Fig. 10C). The uroneural 1 and 2 were formed by fibrous ossification at about 10 and 16 mm TL, respectively (Fig. 10C, D).

Eight accessory cartilages (abbreviation, Ac) of *P. major* were located at the following regions: anterior to the tip of the 22nd neural spine (Ac 1), anterior to the tip of the 12th haemal spine (Ac 2), between the tips of the 12th and 13th haemal spines (Ac 3, 4), posterior to the tip of the 13th haemal spine (Ac 5), posterior to

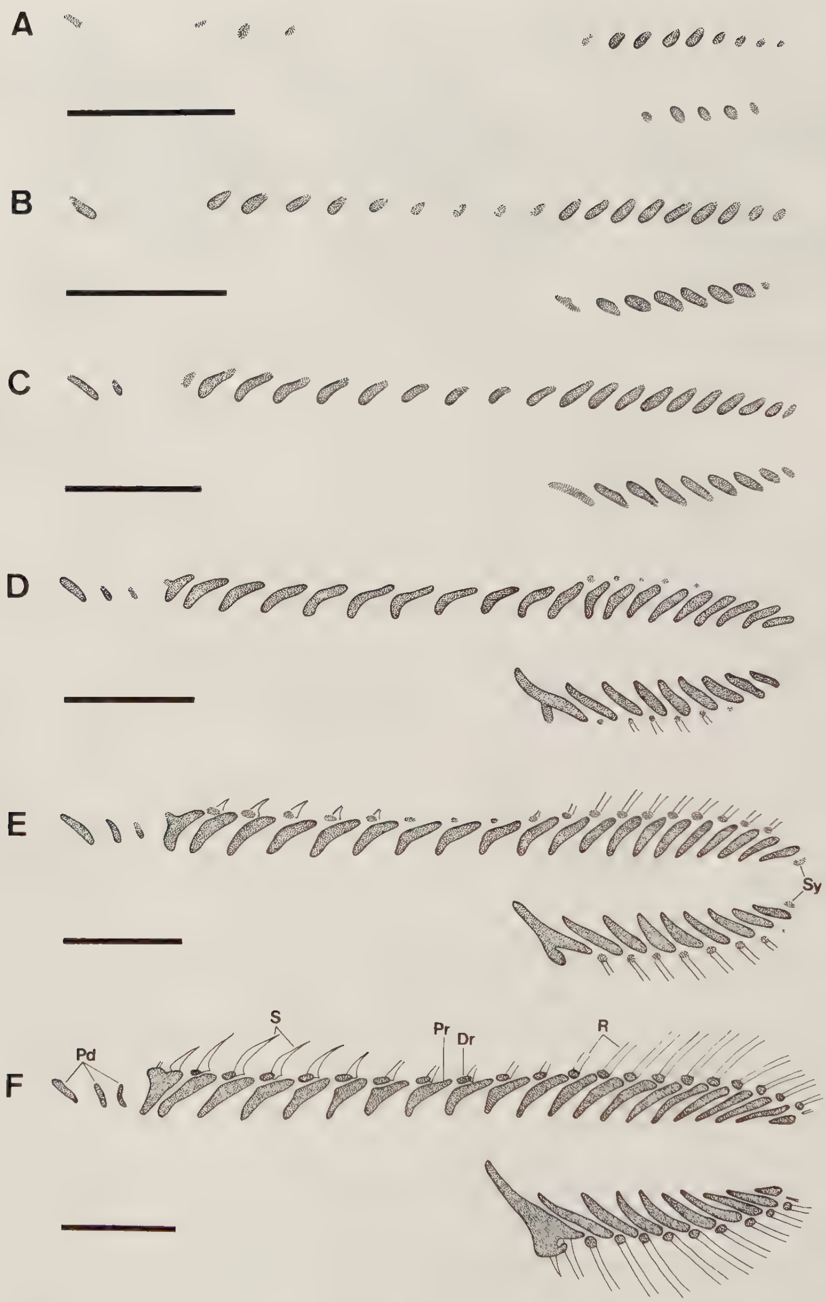
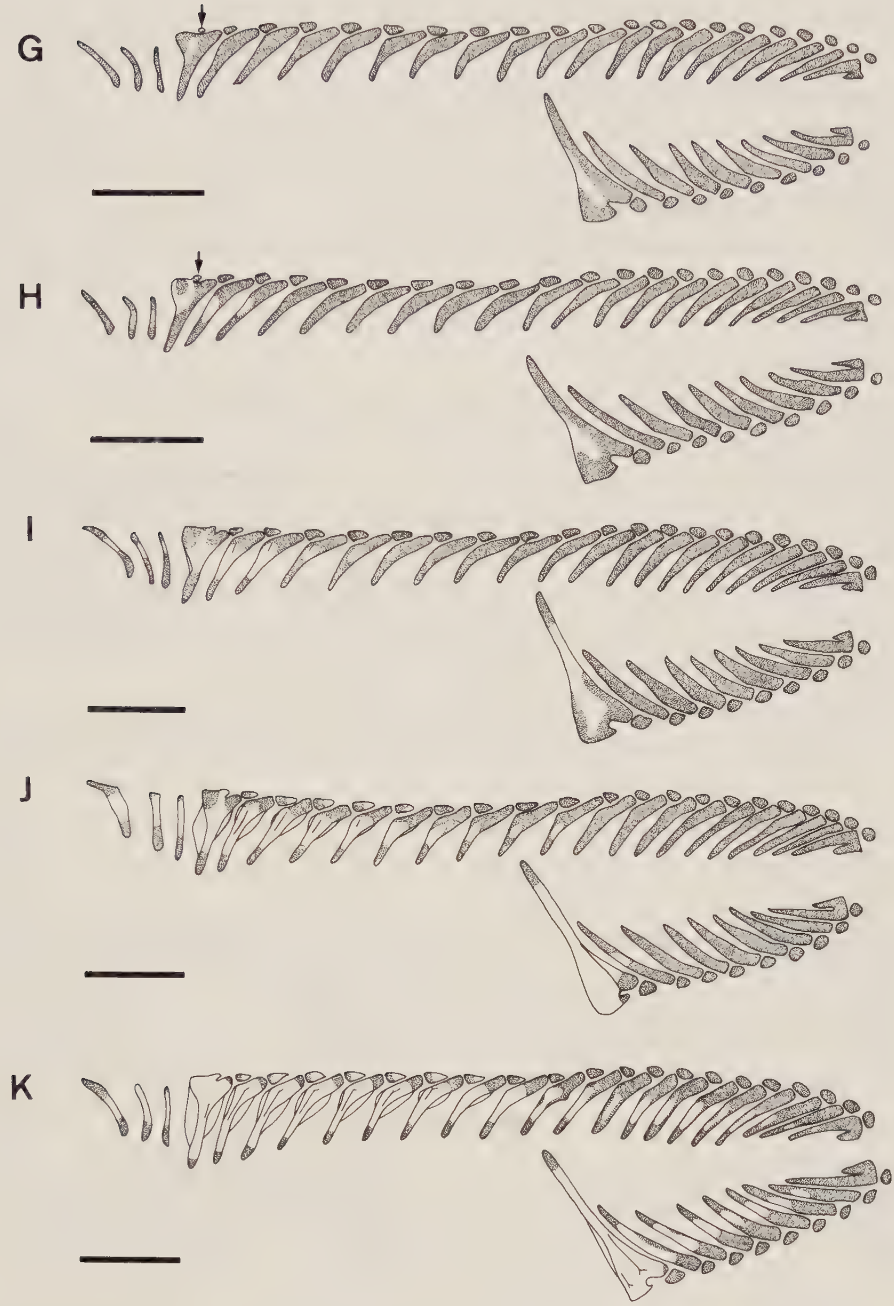


Fig. 11. Development of dorsal and anal fin-supports in *Pagrus major*. A: TL. F: 7.75 mm TL. G: 8.50 mm TL. H: 8.85 mm TL. I: 9.75 mm K. Arrows in G and H show transient first distal radial of dorsal radial; Pd, predorsal; Pr, proximal radial; R, soft ray; S, spine; Sy,



5.80 mm TL. B : 5.85 mm TL. C : 6.40 mm TL. D : 6.85 mm TL. E : 7.25 mm TL. J : 10.0 mm TL. K : 10.5 mm TL. Lateral view. Rays are removed in G– fin-supports. Stippled area, cartilage. Open area, ossified portion. Dr, distal stay. Scale bars indicate 0.5 mm.

the tip of the hypural 5 (Ac 6), on both sides of the posterior tip of the notochord (Ac 7, 8). In some specimens, there were a few small cartilages at the posterior region to the parhypural tip (Fig. 10E).

These accessory cartilages were formed in the following sequence. Ac 3 was the first to be chondrified at about 7 mm TL, and slightly later Ac 5 appeared. Ac 4 and Ac 6 were almost simultaneously formed at about 9 mm TL (Fig. 10B, C). Although the size at which Ac 1 or Ac 2 was formed had a relatively wide range, Ac 2 was first recognized in a 23.0 mm TL and Ac 1 in a 30.5 mm TL specimen. Ac 7 and 8 were the last to be chondrified, by about 70 mm TL (Fig. 10D, E).

Dorsal and anal fin-supports

Three predorsals were chondrified from anterior to posterior between about 6 and 7 mm TL (Fig. 11A-D).

The cartilage formation of the dorsal proximal radials proceeded anteriorly and posteriorly from two centers, around the third and 15th ones, respectively, between about 6 and 6.5 mm TL (Fig. 11A-C).

In the case of the anal proximal radials, the cartilage formation proceeded anteriorly and posteriorly from the fourth or fifth one between about 6 and 6.5 mm TL (Fig. 11A-C).

The first proximal radials of the dorsal and anal fin-supports possessed the anterior cartilaginous process (Fig. 11D, E), which was not separated from the main parts even at the initial appearance.

After the full complement of the proximal radials, the cartilage formation of the dorsal and anal distal radials proceeded in the similar sequence as the proximal radials by about 7.5 mm TL (Fig. 11D, E, F).

Shortly after the appearance of the distal radials, the rudiments of the dorsal and anal fin rays were formed and ossified, and the full complements were accomplished by about 8 mm TL (Fig. 11E, F).

The stay cartilage in the dorsal and anal fin-supports was formed at about 7 mm TL as the separated cartilage mass (Fig. 11E, F) but later fused with the immediate anterior middle-proxiomal radial (Fig. 11G).

Just above the first proximal radial of the dorsal fin-supports, a small cartilage was temporarily formed (arrow in Fig. 11G) and articulated with the second spine. It seemed to be a vestigial distal radial but shortly later fused with the first proximal radial (arrow in Fig. 11H). Such a cartilage was not observed in the first proximal radial of the anal fin-supports.

The ossification of the dorsal and anal fin-supports started simultaneously at the first proximal radials at about 8.5 mm TL (Fig. 11G). The following ossification proceeded posteriorly in the dorsal proximal radials (Fig. 11H, I), and when it reached around the tenth one, the second proximal radial of the anal fin-supports

began to be ossified (Fig. 11J). The posteriormost proximal radials of the dorsal and anal fin-supports were ossified at about 10.5 mm TL (Fig. 11K).

The ossification of the distal radials proceeded from anterior to posterior between about 9 and 15.5 mm TL in the dorsal fin-supports, and between about 10 and 15.5 mm TL in the anal fin-supports, respectively (Fig. 11I, J, K).

The predorsals were ossified between 8.7 and 9.7 mm TL. The anteriormost one was the last to be ossified (Fig. 11H, I).

The stay cartilages of the dorsal and anal fin-supports were ossified at about 25 mm TL (Fig. 12A).

The numbers of the middle radials were considerably variable in both wild and reared specimens, ranging from two to four in the dorsal and from one to four in the anal fin-supports. About one half of the specimens possessed three dorsal middle radials and two anal ones.

The middle radials were ossified from anterior to posterior, on the cartilaginous regions contiguous to the ossified proximal radials (Fig. 12B, C, D). The ossification of the anal middle radials slightly preceded that of the dorsal ones. In specimens smaller than 42 mm TL, no ossification of the middle radials was observed. The smallest specimen with all ossified middle radials was 56.0 mm TL and the middle radials in the largest reared one examined, 72.0 mm TL, were still uncompleted. On the other hand, wild specimens beyond 45 mm TL exhibited all ossified middle radials.

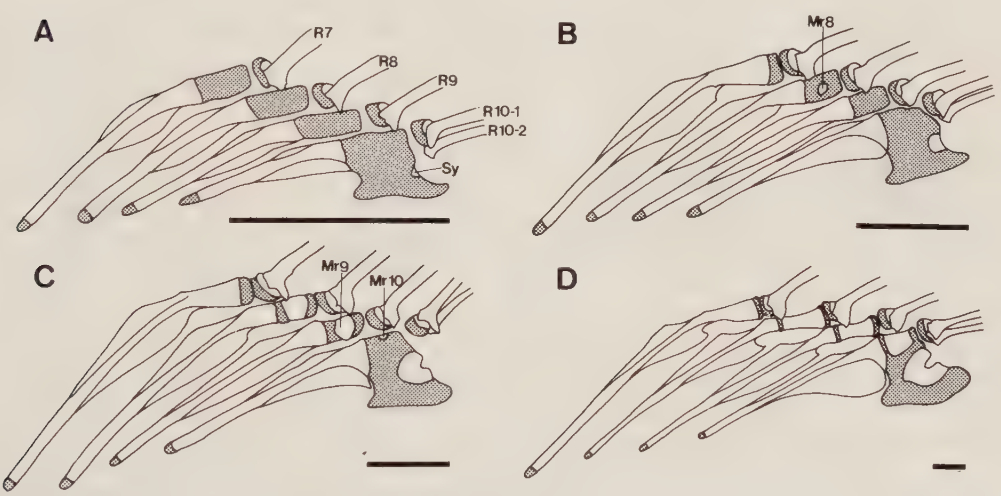


Fig. 12. Development of posterior part of dorsal fin-supports in *Pagrus major*. A: 25.6 mm TL. B: 45.4 mm TL. C: 61.0 mm TL. D: 151.0 mm TL. Lateral view. Stippled area, cartilage. Open area, ossified portion. Mr, middle radial; R, soft ray; Sy, stay. Scale bars indicate 1 mm.

Pectoral fin-supports

In a 2.80 mm TL specimen, the needlelike cleithrum was formed by fibrous ossification, and the fin plate[38] and coracoid-scapula cartilages were present and continuous together. The former cartilage was relatively immature and only one cell thick, but the latter was two or three cells thick. In the coracoid-scapula cartilage, the posterior process of the coracoid was dominant (Fig. 13A).

The fin plate cartilage was creviced in the anterior middle region at about 3.5

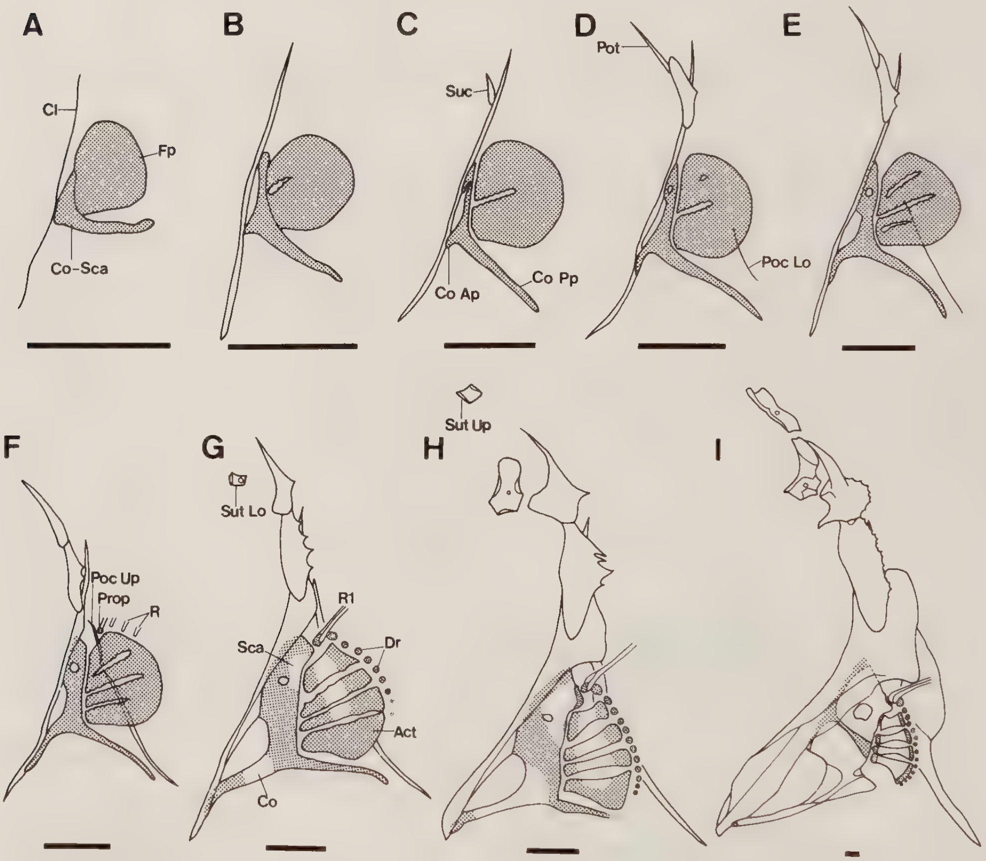


Fig. 13. Development of pectoral fin-supports in *Pagrus major*. A: 2.80 mm TL. B: 3.70 mm TL. C: 5.25 mm TL. D: 5.50 mm TL. E: 5.85 mm TL. F: 6.40 mm TL. G: 8.80 mm TL. H: 10.0 mm TL. I: 35.0 mm TL. Lateral view. Rays except marginal one are removed in G–I. Stippled area, cartilage. Open area, ossified portion. Act, actinost; Cl, cleithrum; Co, coracoid; Co Ap, coracoid anterior process; Co Pp, coracoid posterior process; Co-Sca, coracoid-scapula cartilage; Dr, distal radial; Fp, fin plate cartilage; Poc Lo, postcleithrum lower; Poc Up, postcleithrum upper; Pot, posttemporal; Prop, propterygium; R, soft ray; Sca, scapula; Suc, supracleithrum; Sut Lo, supratemporal lower; Sut Up, supratemporal upper. Scale bars indicate 0.3 mm.

mm TL (Fig. 13B).

The fin plate cartilage was separated from the coracoid-scapula cartilage at about 5 mm TL. The supracleithrum, posttemporal and lower postcleithrum were successively formed by fibrous ossification between about 5 and 5.5 mm TL. The anterior process of the coracoid gradually elongated. The scapular cartilage elongated dorsally and enclosed the scapular foramen (Fig. 13C, D).

The second crevice in the fin plate cartilage appeared dorsally and the third one ventrally on both sides of the first one between about 5.5 and 6 mm TL (Fig. 13D, E).

Between about 6 and 6.5 mm TL, the rudiments of the fin rays began to be formed and ossified from dorsal to ventral, and the upper postcleithrum was formed by fibrous ossification. A small cartilage, propterygium of JESSEN,[39] was formed above the fin plate cartilage (Fig. 13F). Later the propterygium was fused with the inner basal half of the marginal (uppermost) ray and ossified (Fig. 14A-E).

The lower and upper supratemporals were formed by fibrous ossification at about 8.5 and 9 mm TL, respectively (Fig. 13G, H).

The scapula and coracoid were ossified simultaneously at about 8 mm TL. The ossification center of the scapula was behind the scapular foramen, and that of the coracoid was in the basal part of the anterior process (Fig. 13G). The fin plate cartilage was completely divided by three crevices into four actinosts by about 9 mm TL. The ossification of the actinosts occurred from dorsal to ventral between about 8.5 and 9.5 mm TL (Fig. 13G, H).

The full complement of the pectoral fin rays was accomplished by about 9.5 mm TL. The distal radials were chondrified from dorsal to ventral, following the appearance of the fin rays (Fig. 13G), and their full complement was accomplished by

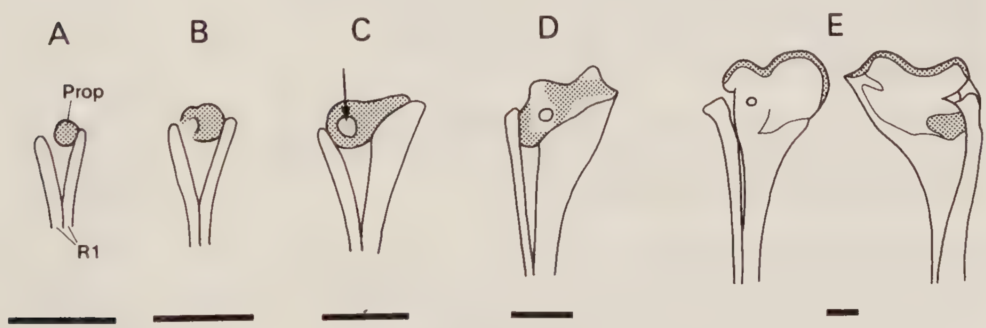


Fig. 14. Development of propterygium of pectoral fin-supports in *Pagrus major*. A: 6.25 mm TL. B: 6.85 mm TL. C: 9.75 mm TL. D: 18.7 mm TL. E: 35.0 mm TL. Dorsal view (A-D). Dorsal (left) and ventral (right) views (E). Arrow in C shows canal throgh propterygium. Stippled area, cartilage. Open area, ossified portion. Prop, propterygium; R, soft ray. Scale bars indicate 0.1 mm.

about 11 mm TL. The distal radials were not ossified even in the largest specimen examined, 151.0 mm TL.

The cartilaginous posterior process of the coracoid was gradually shortened and almost disappeared between about 8.5 and 13 mm TL (Fig. 13G, H, I).

Pelvic fin-supports

The membraneous fin fold of the pelvic fin appeared at about 6 mm TL and the basipterygium was chondrified at about 6.5 mm TL (Fig. 15A). The basipterygium gradually elongated and began to be ossified at the posterior region at about 8.5 mm TL (Fig. 15B), and the anterior tip reached the cleithrum at about 10 mm TL (Fig. 15C).

The rudiments of the fin rays began to be formed and ossified from outer to inner at about 7 mm TL and their full complement was accomplished by about 8.5 mm TL.

A small cartilage, metapterygium of SEWERTZOFF,[40] was formed behind the basipterygium by about 9 mm TL (Fig. 15B). The metapterygium was fused with the ventral basal half of the innermost fin ray and ossified later (Fig. 15D).

More detailed sequences of the cartilaginous-osteological development in reared specimens are shown in Figs. 16 and 17.

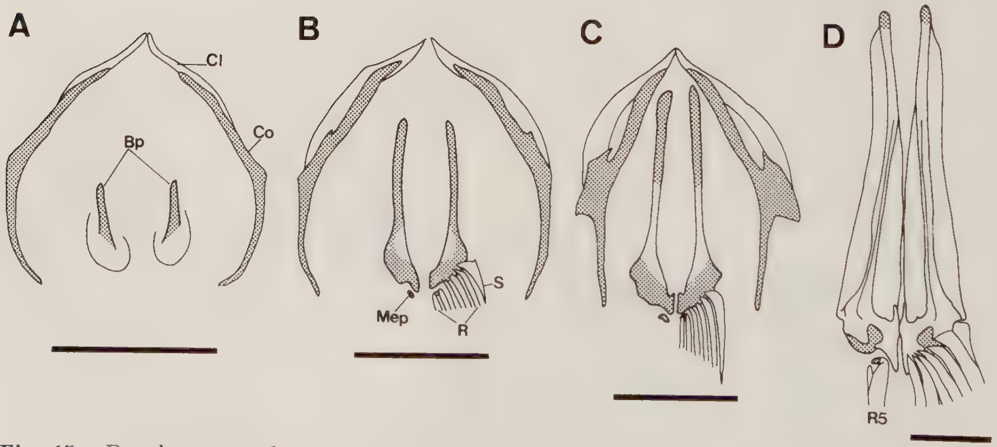


Fig. 15. Development of pelvic fin-supports in *Pagrus major*. A: 6.85 mm TL. B: 8.85 mm TL. C: 9.75 mm TL. D: 20.0 mm TL. Ventral view. Pectoral fin-supports are also shown in A-C. Rays of right body side are removed in B and C and innermost ray fused with metapterygium is shown in D. Stippled area, cartilage. Open area, ossified portion. Bp, basipterygium; Cl, cleithrum; Co, coracoid; Mep, metapterygium; R, soft ray; S, spine. Scale bars indicate 0.5 mm.

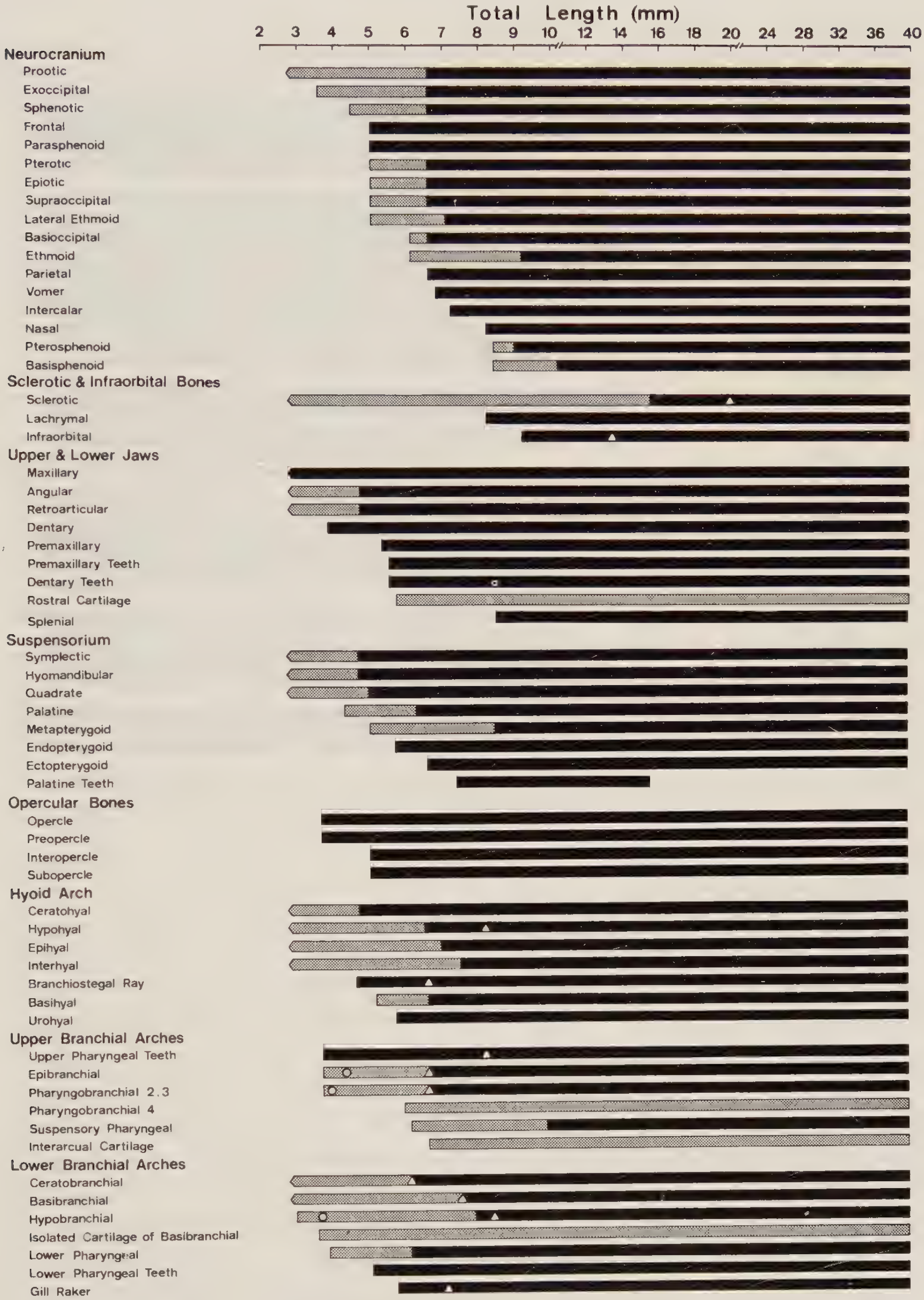


Fig. 16. Developmental sequence of head skeleton in reared *Pagrus major*. Stippled bar, cartilaginous state. Solid bar, ossified state. ○, full complement of cartilages. △, full complement of ossified structures.

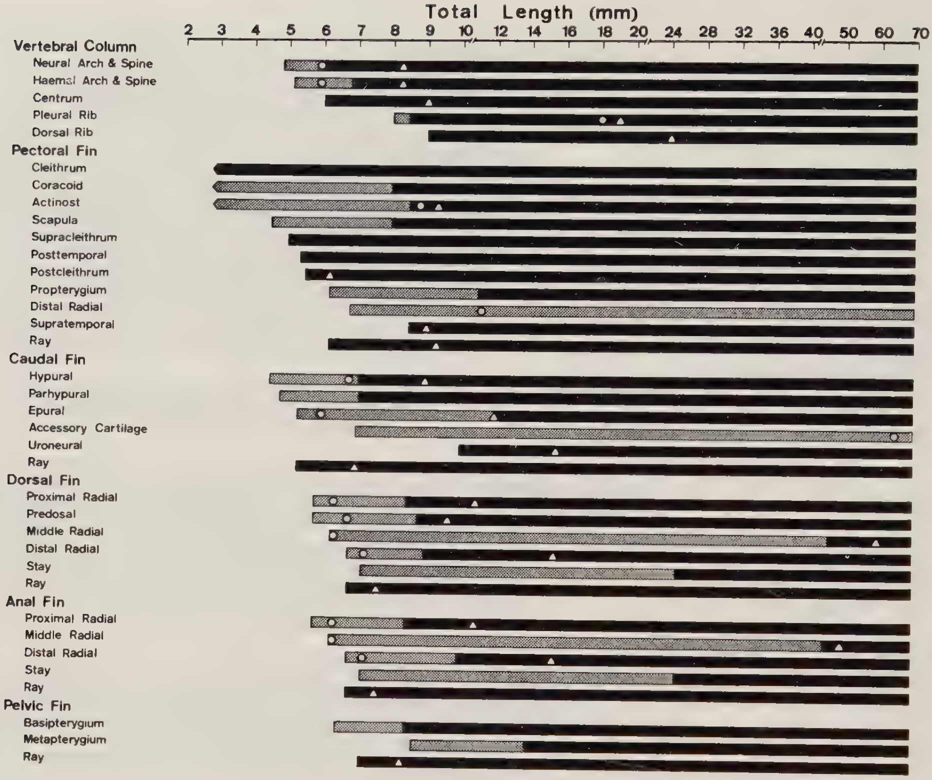


Fig. 17. Developmental sequence of vertebral column and fin-supports and rays in reared *Pagrus major*. Stippled bar, cartilaginous state. Solid bar, ossified state. Symbols are the same in Fig. 16.

Discussion

Some comments should be given about the development of the cartilaginous-osteological structures of *P. major* while comparing with previous literature on the same species or other teleosts.

Fish bones can be generally divided into two types: one is preformed in cartilage (cartilage bone) and the other directly formed by fibrous ossification (dermal bone and membrane bone). Misinterpretation about bone origin has been frequently made even in some literature in which the double staining method for bone and cartilage of DINGERKUS and UHLER[26] is used. For example, LAU and SHAFLAND[41] described the "cartilaginous" uroneural, ray, maxillary, cleithrum, urohyal, opercle and so on. All these bones, however, are directly formed by fibrous ossification, and are not preformed in cartilage. KOHNO *et al.*[15] also misunderstood the origin of the centrum as cartilage, as KOHNO and TAKI[27] pointed out. Such misinterpreta-

tion probably happened because alcian blue does not only stain cartilage but also fibrous tissues or decalcified bony tissues, although cartilages are stained more deeply than other tissues. Therefore, distinguishing between cartilage and other tissues should be made by careful confirmation of cartilage cells under a microscope or more correctly by histological observations.

The development and structures of the branchial arches in *P. major*, except for a little difference, are almost similar with *Anisotremus virginicus* reported by POTTHOFF *et al.*[28] They described the presence of the tooth plate on the epibranchial 2 in only juveniles, but such structure was not recognized during the ontogeny of *P. major*. KOHNO et al.[15] illustrated the branchial arches of a larval *P. major*, but they seem to have mislabeled the pharyngobranchials.

The sequence of the cartilaginous formation of the neural and haemal arches along the notochord is considerably different among several teleosts. In *Clupea pallasii*, both neural and haemal arches start to develop anteriorly from a point of the penultimate centrum, and, shortly later, posteriorly from just posterior to the head.[42] In *Xiphias gladius*, both neural and haemal arches are successively formed from anterior to posterior.[43] In *Scombrolabrax heterolepis*, the neural arches develop at the anteriormost portion of the notochord and the posterior portion to the center of the notochord, and the heamal arches develop from the center at middle of the future anal fin.[44] In *A. virginicus*, the neural arches first appear anteriorly and at the center of the notochord, and the haemal arches also at the center of the notochord.[28] In the two species of *Morone*, the neural arches appear from anterior to posterior, while the haemal arches develop from a center anteriorly and posteriorly.[45] The sequence of the cartilaginous formation of the neural and haemal arches in *P. major* fundamentally resembles those of *S. heterolepis*, *A. virginicus* or *Morone*, although the formation of the first neural arch and completion of the central abdominal neural arches occurs later in the former.

In *P. major*, cartilage cell mass of the future neural arch first appears at different regions, e. g. just on the notochord or on the dorsal side of the spinal cord, in contrast to the parapophysis and haemal arch cartilages which uniformly appear beneath the notochord. The first to third neural arches are initially formed on the notochord, the fourth to 14th ones on the dorsal side of the spinal cord and the 15th to 18th ones simultaneously on both the notochord and the dorsal side of the spinal cord. Although such detailed observation has not been made in any other fishes, it may be interesting to relate the induction mechanism of the vertebrae probably by the notochord and spinal cord, as indicated in other vertebrates.[46]

The ossification sequence of the neural and haemal arches is somewhat simpler than the chondrification sequence. In *P. major*, ossification proceeds from the second neural arch anteriorly and posteriorly, preceding the formation of the cen-

trum in the respective vertebra. The earlier ossification of the neural and haemal arches than the centrum is observed in *Archosargus rhomboidalis*,[32] *Pneumatophorus diego* (=*Scomber japonicus*),[47] *Channa argus*[48] and *Salvelinus*,[49–52] while it is reported that the neural and haemal arches are later ossified than the centrum in *Cyprinus carpio*[48] and *Thunnus atlanticus*.[53]

In relatively lower teleosts such as *C. pallasii*,[42] *C. carpio*,[48] *Opisthonema oglinum*,[54] *Plecoglossus altivelis*[55] and *Pollichthys mauli*,[56] the formation of the centrum is delayed in the anterior portion along the notochord. On the other hand, in many perciform fishes, the formation of the centrum proceeds from anterior to posterior except the posterior end.[32,41,43,45,47,48,53,57,58] TAKASHIMA[14] reported the same sequence of formation of the centrum in *P. major*. In my observations, however, the second centrum is the first to be formed and the anteriormost centrum appears later than several posterior ones. Similar observation is made by POWELL and GORDY[59] in their illustration of 5.1 mm SL *Leiostomus xanthurus*, which indicates the delay of the formation of the first centrum than several posterior ones. In *A. virginicus*,[28] *C. undecimalis*,[41] the two species of *Morone*[45] and *T. atlanticus*,[53] it is suggested that the urostyle and preural centrum 2 and 3 are formed from posterior to anterior. On the other hand, in *P. major*, except for the urostyle, the preural centrum 2 and 3 appear from anterior to posterior. The sequence of the chondrification and ossification of the vertebral column is considerably variable in many teleosts, as shown here, although to some extent similarity of the sequence between related species is recognizable. Further detailed and extensive studies will be required to generalize the sequence pattern in many teleosts.

The formation process of the cylindrical centrum surrounding the notochord is also different among species. In *P. diego*[47] and *T. atlanticus*[53] belonging to the Perciformes, the ossification of the centrum simultaneously begins at two regions surrounding the basal parts of both neural and haemal arches, and later these rudiments of the centrum became fused at the lateral side of the notochord. On the other hand, the entire cylindrical centrum stains evenly at onset of stain uptake in *Morone*.[45] Different from these examples, the ossification of the first to 21st centra of *P. major* begins at the region surrounding the basal part of the neural arch and proceeds ventraly, and the ossification of the 22nd and 23rd centra (preural centrum 3 and 2) begins at the region surrounding the basal part of the haemal arch and proceeds dorsally.

KOHNO *et al.*[15] mentioned that the urostyle of *P. major* rises from two separate elements, most probably the ural centrum 1 and 2. In my observations, however, such evidence could not be detected during ontogeny of *P. major*, and the urostyle seems to be formed as an independent element from initial appearance without any fusion. The independent formation of the urostyle is also reported in *Paralichthys*

olivaceus.[60]

It is suggested by some authors that the centrum of fishes is derived from two origins (chordacentrum and autocentrum), especially in relatively lower teleosts (see, LAERM[61]). At first, the internal chordacentrum is formed by calcification of the fibrous sheath, and later the external autocentrum is added by ossification in the perichordal mesenchyme. FRANCOIS[62,63] reported that the chordacentrum gets thinner in its mid portion, then reduces or disappears with growth. Although the early formation of the centrum by both the chordacentrum and autocentrum is speculated in all teleosts, FRANCOIS[62] could not show a distinct chordacentrum in larvae and juveniles of relatively advanced teleosts, e. g. *Mugil*, probably for very early degeneration of the fibrous sheath except the intervertebral regions. The present study indicates that the thin chordacentrum definitely exists in an advanced teleost, *P. major*, as well as lower teleosts. Unfortunately, further detailed observations could not be performed. Electron microscopic investigation will be necessary in this field.

It is indicated in many teleosts that the cartilage formation of the caudal skeleton occurs at an early stage (e. g. HOUDE and POTTHOFF,[32] GWYN,[42] FRITZSCHE and JOHNSON,[45] BALON,[49,51] POTTHOFF[53,58]). GWYN[42] confirmed that the hypural cartilages appear earlier than other elements of the caudal skeleton and vertebral column in *C. pallasii*. The present study showed that the hypural 1 is the first to be chondrified at about 4.5 mm TL and the parhypural and hypural 2 are the second in *P. major*. HOUDE and POTTHOFF[32] described a 3.4 mm NL (3.5–3.6 mm TL) larva having the parhypural, hypural 1 and 2 in *A. rhomboidalis*, closely related to *P. major*. It is likely that the cartilage formation of the caudal skeleton in the former species starts considerably earlier than the latter.

Previously, there was little fragmentary knowledge about the accessory cartilages of the caudal fin-supports, for example, in *Eleutheronema tetradactylum*,[64] and the four species including *Maurolicus mueleri*.[65] Information on these cartilages has been recently increasing.[28,41,45,66] The distribution pattern of the accessory cartilages in the caudal fin-supports of *P. major* resembles that of *Morone*,[45] except for some of the following differences: (1) the former always has two cartilages (Ac 3, Ac 4) between the proximal tips of the haemal spines of the preural centrum 2 and 3 but the latter one or two depending on specimens. (2) no cartilage corresponding to Ac 7 and Ac 8 in the former is described in the latter. (3) one half of specimens of *Morone saxatilis* has a cartilage anterior to the anteriormost epural but such a cartilage is not recognized in the former. Because Ac 7 and Ac 8 are the last to be formed in all cartilaginous-osteological structures of *P. major*, they may be still undifferentiated throughout the size range examined in *Morone*. POTTHOFF *et al*.[28] indicated that three accessory cartilages between the proximal tips

of the haemal spine of the preural centrum 3 and the parhypural (correspond to Ac 3, 4 and 5 in *P. major*) gradually change their position during development. Moreover, they described the presence of the separate articular cartilages at the proximal tips of the haemal spine of the preural centrum 2 and the parhypural in adults. In *P. major*, both the distinct positional change of the accessory cartilages during development and the presence of the separate articular cartilages were not observed up to 151.0 mm TL. Without doubt, the number and distribution pattern of the accessory cartilages are characteristic of species or a certain taxon, and they probably have a large systematic utility, as shown by JOHNSON.[66]

In regard to the development of the first dorsal proximal radial in *P. major*, KOHNO *et al.*[15] and KOHNO and TAKI[27] indicated its derivation from two cartilaginous pieces. Similar observations were made in *A. virginicus*[28] and *Morone americana*.[45] In my observations, however, the usual development did not show the fusion of two elements or the formation of foramen within the first proximal radial described by KOHNO *et al.*[15] (Fig. 2C and D). Several larvae having a relatively large process or separated cartilage from the first proximal radial were observed, but they seem to become abnormal juveniles with the malformed first proximal radial or the additional proximal radial just anterior to the first one, which are frequently found in reared specimens (see Chapter Ⅲ-2). In spite of the lack of fusion during usual ontogeny, there is no doubt that the first dorsal proximal radial has been originally derived from at least two elements through evolution, as evidenced by the two distal radials temporarily possessed by this proximal radial, as shown in the present study.

In *A. virginicus*,[28] *Xiphias gladius*,[43] *S. heterolepis*,[44] *T. atlanticus*,[53] *Coryphaena hippurus* and *C. equiselis*,[58] it is suggested that the proximal and distal radials in the dorsal and anal fin-supports are formed by segregation from a rod-shaped pterygiophore cartilage. In *P. major*, the proximal and distal cartilages were formed as respective independent cartilages from initial appearance, as mentioned by KOHNO and TAKI[27] in same species and BALART[60] in *P. olivaceus*. Such difference in observations may not be regarded as species difference and is presumably raised from misinterpretation in the former species.

KOHNO *et al.*[15] suggested that the "pectoral condyle" of *P. major* may be homologous with the distal radials. However, their "pectoral condyle" is not the structure represented by the same term by STARKS[67] but should correspond to the propterygium of JESSEN.[39] The propterygium in the actinopterygians has general characteristic of the fusion with the basal half of the marginal (uppermost) ray and the penetration of nerve and vessel. Although the propterygium of *P. major* lacks the process of separation from the fin plate cartilage, different from that of *Engraulis* and *Oncorhynchus*[27] and *Clupea* (illustrated by GOODRICH[68]), it is not homol-

ogous with the distal radials. POTTHOFF *et al.*[28] and POTTHOFF and KELLEY[43] mentioned that the "distal radial" supporting the uppermost ray originates from the scapular cartilage in *A. virginicus* and *X. gladius*, respectively. They presented no evidence showing its segregation from the scapular cartilage and such developmental process seems to be unlikely.

Figures 18 and 19 summarize the counts of noticeable changes in the osteological development, for example, the initial appearance of cartilage, the full complement of cartilages, the beginning of ossification and the full complement of ossified structures for respective elements, based on Figs. 16 and 17. The counts were made in each 0.5 mm size range (less than 10 mm TL), each 1 mm (10 to 20 mm

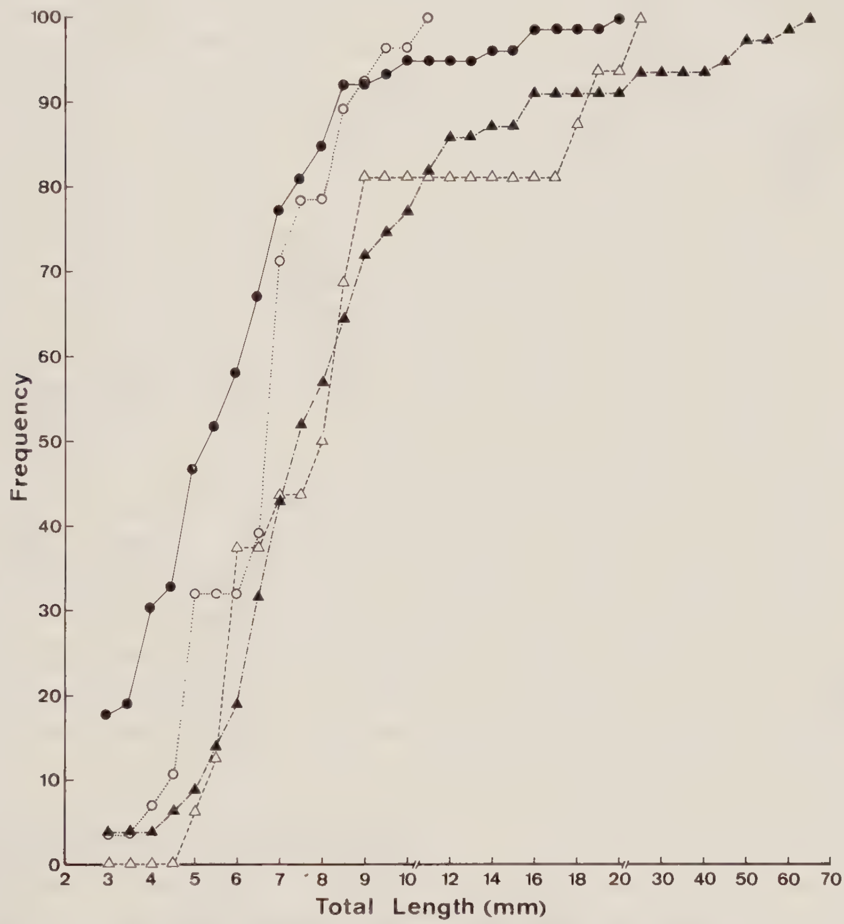


Fig. 18. Relationship between counts of osteological developmental changes (cumulative percentage) and total length for four skeletal groups in reared *Pagrus major*. ○, neurocranium. ●, head skeleton except neurocranium. △, vertebral column. ▲, fin supports and rays.

TL) or each 5 mm (beyond 20 mm TL). This treatment may be useful for total understanding of the osteological development as an aggregation of qualitative changes.

Figure 18 shows the cumulative percentage of the counts of progressed changes against total length for four skeletal groups, the neurocranium, head skeleton except the neurocranium, vertebral column and fin-supports and rays. It may show the "developmental degree of osteological characters". All lines pass through a sigmoid pattern. In the head skeleton except the neurocranium, the value at first-feeding stage (about 3 mm TL) is the highest of four skeletal groups, about 20%, and it reaches about 95% at 10 mm TL. In the neurocranium, the value at first-feeding stage is considerably as low as the vertebral column and fin-supports and rays, but it rapidly increases and reaches almost 100% at 10 mm TL. On the other hand, the vertebral column and fin-supports and rays are formed at relatively late stages. The cumulative percentages of changes of both are zero or low at first-feeding stage and not more than about 80% at 10 mm TL. The value of the fin-supports and rays gradually increases up to 60-70 mm TL, and the delay of completion of the vertebral column is mainly due to the late appearance of its appendages, the pleural and dorsal ribs.

At first-feeding stage, the cartilaginous-osteological elements consist of parts of the neurocranium (trabecula, parachordal and auditory capsule cartilages), the jaws (maxillary and Meckel's cartilage), the suspensorium (quadrate and hyomandibular-symplectic cartilages), the hyoid arch (hypohyal, ceratohyal-epihyal and interhyal cartilages), the lower branchial arches (basibranchial, ceratobranchial and hypobranchial cartilages), the pectoral fin-supports (cleithrum, coracoid-scapula and fin plate cartilages) and the sclerotic cartilage. The jaws, suspensorium, hyoid arch and lower branchial arches function in opening and closing of the mouth, and expanding and narrowing of the buccal and branchial cavities. These functions are indispensable for swallowing the water which contains food organisms. The early formation of these skeletal elements points to with their most fundamental importance for larval survival. At this stage, respiration mostly depends on cutaneous respiration (YAMASHITA[3] recognized the anlagen of gill filament at this stage of *P. major*. In my observations, however, larvae at first-feeding stage have no distinct gill filament). Several days later, the gill filaments begin to be formed, and the above functions also have an important role for respiration. At first-feeding stage, the brain is protected by the subdermal space filled with liquid, although the neurocranium is only formed at the basal part. As the subdermal space gradually disappears, the dorsal and lateral faces of the neurocranium are chondrified or ossified. These changes are almost completed by about 10 mm TL. The relatively early completion of the neurocranium seems to be concerned with the important function of protecting the brain for larval survival. In the fin-supports, only a

part of the pectoral fin-supports is formed at first-feeding stage. The cleithrum supports the sternohyoideus which functions in pulling the lower jaw (see Chapter II-2) and the coracoid-scapula cartilage attached to the fin plate cartilage. The fin plate cartilage is relatively large, functioning as propulsion by fanning, and assists the undulation of the caudal region. Kohno *et al.*[15] mentioned that the fin fold larvae of *P. major* only stay still in stagnant water or orient themselves at the same position in slow-flowing water, without showing any appreciable movement of the body. Their observations, however, probably seem to be made on larvae which are enfeebled or with a full stomach. Fukuhara and Kishida[21] and my observations revealed that the fin fold larvae of *P. major* show relatively active swimming behavior for seaching and attacking food organisms, although swimming is still intermittent. The caudal fin-supports are the second to be formed among all the fin-supports. As the caudal fin-supports and rays develop, they can increase the strength of tail beats. The vertebral column and dorsal, anal and pelvic fin-supports appear at later stages. The vertebral column not only gives firmness to the body axis, its segmentation also gives flexibility in bending the fish body. Such functions can also increase swimming ability. The dorsal, anal and pelvic fin-supports articulated with rays are functional for braking, changing of swimming direction, etc., beyond late postlarvae or juveniles in which the fin fold gets to disappear. The fin plate cartilage of the pectoral fin-supports separates into four actinosts and becomes relatively smaller as the larvae grow, so that the pectoral fin-supports and rays decrease their early function in active propulsion and starts to operate for braking, controlling the vertical course of swimming and so on.

Figure 19 shows the histogram of developmental changes of all osteological elements except the scale against total length. Fukuhara[17] reported that the scale of *P. major* appears between 7.5 and 8.0 mm in body length (BL) and it covers the whole lateral surface of the fish body by about 15 mm BL. Such developmental process of the scale is within the range where those of other osteological elements occurs, as shown here. The total number of osteological developmental changes except the scale amounts to 199. By first-feeding stage (about 3 mm TL), 18 changes have already occurred. In these changes, cartilaginous ones occupy 16. Cartilaginous changes continuously occur beyond 3.5 mm TL and ossificatory ones rapidly increase beyond 4.5 mm TL. By 10 mm TL, 177 (about 90%) changes have occurred. The cartilaginous-osteological development in reared specimens is completed by about 70 mm TL, characterized by the formation of Ac 7 and 8 of the caudal skeleton and the ossification of the middle radials of the dorsal and anal fin-supports. In this histogram, several peaks of osteological developmental changes can be recognized. These will be discussed in Chapter IV, in relation to saltatory development and ontogenetic intervals in fish ontogeny.

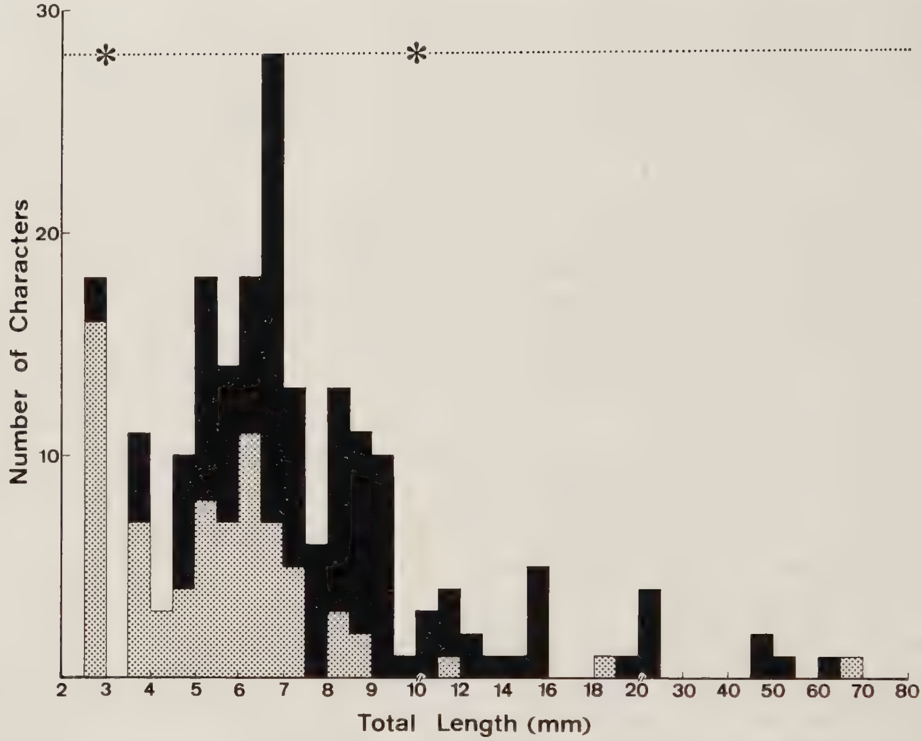


Fig. 19. Occurence distribution of ostelogical developmental changes against total length in reared *Pagrus major*. Stippled, chondrification. Solid, ossification. *, boundary between periods.

II. DEVELOPMENT OF SKELETAL MUSCLES

The myotomal muscle, the most important source of propulsive power in swimming, is spread over the trunk and caudal regions, and the largest one in the skeletal muscles of fish. The head muscle consists of many relatively small muscles which attach and operate the respective head skeletons by their adduction and abduction. They are particularly closely related to feeding and respiration. The fin muscles also consist of several muscles which attach and operate their respective fin-supports and rays. They function partly for propulsion and partly for maneuverability during swimming.

1. Myotomal Muscle

This section is separated into two divisions. The first is concerned with the qualitative changes of the myotomal muscle, i.e., the differentiation of fiber types

which have respectively specific functions in swimming performance. The second is concerned in the quantitative changes of the myotomal muscle fibers, i.e., the morphometric growth dynamics of respective fiber types.

1-1. Fiber Types and Fiber Differentiation

Recently, information on the structure and function of fish muscles has been rapidly increasing (Bone,[69] Johnston,[70] Endo and Kimura,[71] Tsukamoto,[72] Gill *et al.*,[73] etc.). In most fish, the myotomal muscle consists of two major zones, red and white muscles. The red muscle is composed of small diameter fibers which rely on an aerobic form of metabolism, and the white muscle is composed of large diameter fibers which operate principally by anaerobic glycolysis. Functionally, the former is mainly implicated in sustained slow swimming and the latter implicated in bursts of violent activity. In addition to these two types, the intermediate or pink muscle has been observed in many fish. Johnston *et al.*[74] suggested that this muscle is associated with the intermediate swimming speed in the carp.

In spite of many works about the myotomal muscle of adult fishes, there is few literature concerning the development of fiber types in teleost larvae in relation to swimming performance. Generally, newly hatched larvae from small pelagic eggs are at a much less advanced condition than those hatched from demersal eggs.[10] Therefore, the early development of the myotomal muscle may influence the initial feeding and survival of pelagic larvae. This division was devoted to clarify the types of the myotomal muscle fibers and to pursue their differentiation in *P. major*.

Most parts of this division have already been presented by Matsuoka and Iwai.[75]

Materials and methods

Specimens

Specimens examined were through laboratory-produced series from newly hatched larvae to juveniles up to 20 mm TL, reared in the Fisheries Research Station, Kyoto University in 1981. Incubation and rearing temperatures ranged from 17.1 to 22.6℃. Two wild specimens, caught in Obama Bay (Fukui Prefecture) in 1981, were examined to distinguish the muscle fiber types. Total length was measured prior to preservation.

Histology

Larvae fixed in Bouin's solution were embedded in methacryl resin. Sections were cut at 2 μm and stained by Harris' hematoxylin and eosin.

Histochemistry

Larvae and juveniles anesthetized by MS 222 were frozen immediately in dry ice(solid carbon dioxide)-iso-pentane, as a whole body or decapitated one depending on size. In the case of large wild specimens, the muscular blocks near the horizontal septum were dissected out from three parts of the body, the anterior trunk region, anal region and posterior caudal region, before freezing. These samples were preseved in dry ice for several weeks until examination. Frozen transverse sections were cut at 8-10 μm, mounted on glass coverslips and dried in desiccator at −20°C for one night. Histochemical reactions were examined by the following methods:

(1) Lipid. Sections were stained with a saturated solution of Sudan Black B in 70% ethyl alcohol for 60 min at room temperature, and mounted in glycerin-gelatin.

(2) Succinate dehydrogenase (SDH). Sections were stained by the method of BARKA and ANDERSON.[76] Sections were incubated for 60-90 min at room temperature, and mounted in glycerin-gelatin.

(3) NADH dehydrogenase (NADHD). Sections were stained by the method of BARKA and ANDERSON.[76] Sections were incubated for 30 min at room temperature, and mounted in glycerin-gelatin.

(4) Phosphorylase (PhR). Sections were stained by the method of TAKEUCHI and KURIAKI.[77] Sections were incubated for 60 min at room temperature, and mounted in iodine-glycerin.

Measurement of fiber diameter

Outlines of frozen transverse sections of muscle fibers were drawn on the paper under a binocular microscope with camera lucida. After the drawn areas were measured by a planimeter, the diameters of fibers were calculated as the diameters of the circles.

Results

Fiber types

Three main types of muscle fibers, red, pink and white, were observed in the myotomal muscle of large wild specimens of *P. major*.

The red fibers were observed as a thin lateral superficial sheet just beneath the skin. They were uniformly small in diameter. The mean diameter was 17.1 μm in a specimen of 68.4 mm TL (Fig. 20). These fibers stained intensely for SDH, NADHD, PhR and lipid (Fig. 21A, B).

Lying beneath the red fiber zone was the pink fiber zone. In transverse sections, these fibers increased inward in diameter, but some small diameter fibers were scattered throughout the pink fiber zone (Fig. 21A, B). Staining reactions for

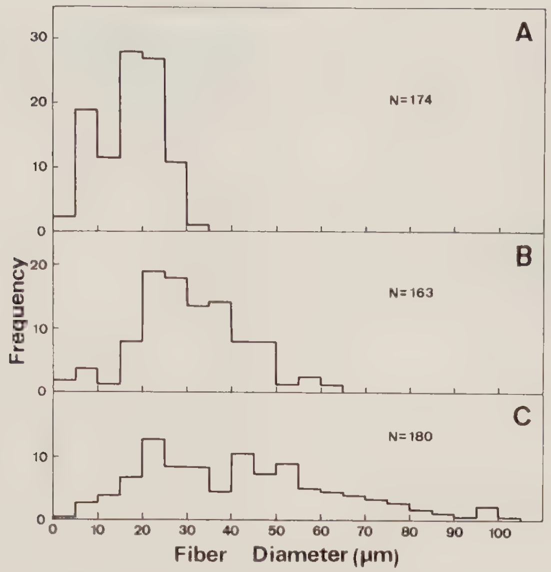


Fig. 20. Frequency distribution of fiber size of myotomal muscle in *Pagrus major*, 68.4 mm TL. A: red fibers. B: pink fibers. C: white fibers. Numbers refer to number of muscle fibers measured.

SDH, NADHD, PhR and lipid in these fibers became gradually lighter inward. The mean diameter was 30.9 μm in a specimen of 68.4 mm TL (Fig. 20). Internally, the pink fiber zone was deeply inserted to the white fiber zone as a thin layer along the horizontal septum and formed one or two small sheets showing intense reactions for SDH, NADHD, PhR and lipid near the centrum (Fig. 21C).

Between the zones of the red and pink fibers, there were fibers of very small diameter, characterized by very weak or negative staining for SDH, NADHD, PhR and lipid (Fig. 21A, B). These fibers ranged 3.6–14.1 μm and averaged 7.9 μm in diameter in a specimen of 68.4 mm TL.

The white fibers occupied most part of the myotomal muscle. This zone was situated beneath the pink fiber zone, but the boundary between the zones of the pink and white fibers was unclear. The mean diameter was 42.4 μm in a specimen of 68.4 mm TL (Fig. 20). The large white fibers and small white fibers were intermingled throughout the white fiber zone, and showed a mosaic appearance in transverse sections. All the white fibers scarcely stained for SDH, NADHD and lipid (Fig. 21A, B, C), but light staining was observed for PhR in the small white fibers (Fig. 21D).

The relative proportion of the red fiber zone and pink fiber zone differed according to the region of fish body. In the anal region, the red fiber zone was nearly the same as the pink fiber zone in thickness (Fig. 21E). In the anterior trunk

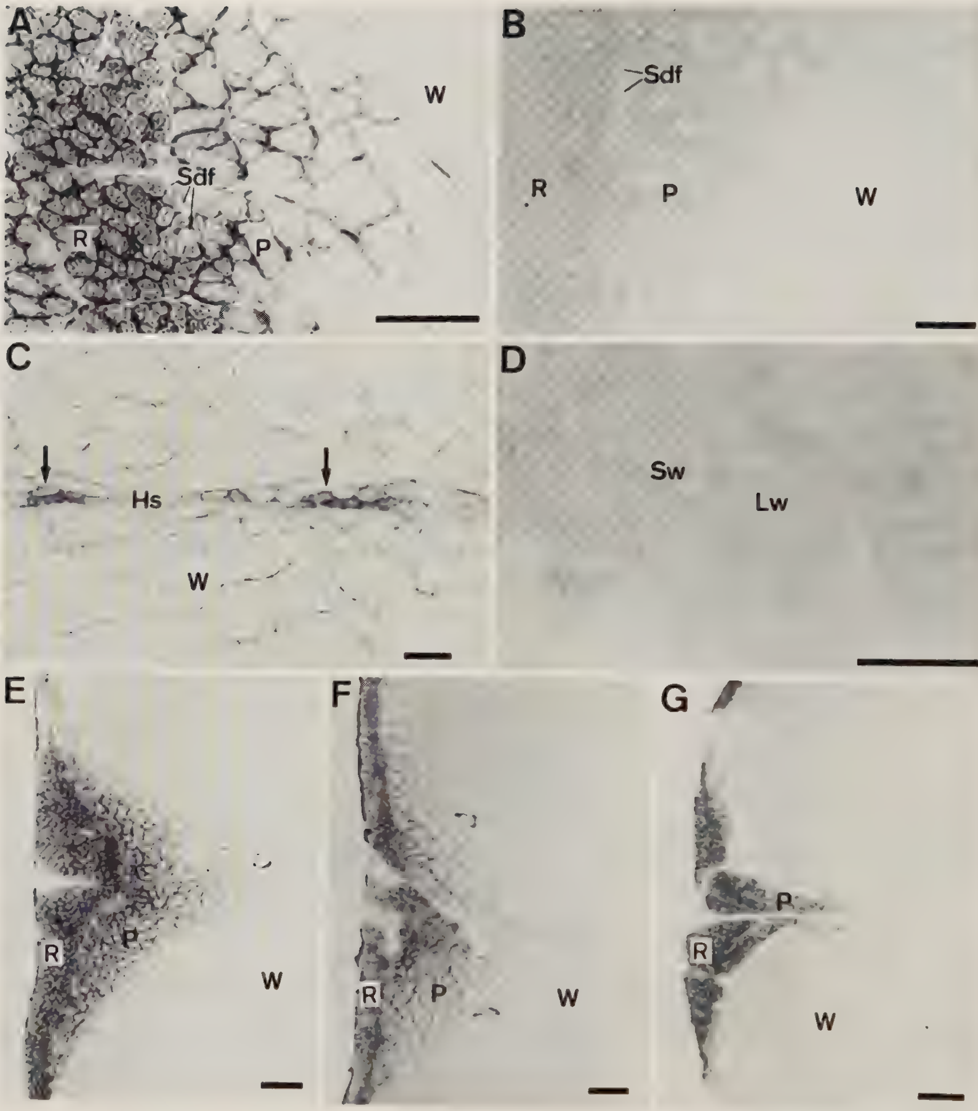


Fig. 21. Photomicrographs of myotomal muscle fibers in *Pagrus major*, 68.4 mm TL (A, E, F, G) and 103.8 mm TL (B, C, D). A: transverse section stained for NADHD. B: transverse seciton stained for lipid. Note a mosaic arrangement of pink and white fibers. C: transverse section stained for NADHD, showing intense staining of pink fibers (arrows) along horizontal septum. D: transverse section stained for PhR, showing light staining of small white fibers and negative staining of large white fibers. E: transverse section in anal region stained for NADHD. F: transverse section in anterior trunk region stained for SDH. Note thin red fiber zone. G: transverse section in posterior caudal region stained for NADHD. Note thin pink fiber zone. Hs, horizontal septum; Lw, large white fibers; P, pink fibers; R, red fibers; Sdf, very small diameter fibers; Sw, small white fibers; W, white fibers. Scale bars indicate 100 μm (A, B, C, D) and 200 μm (E. F, G).

region, the red fiber zone became thinner, but the pink fiber zone showed a similar thickness to that in the anal region (Fig. 21F). On the other hand, in the caudal region, the red fiber zone was remarkably thicker than the pink fiber zone (Fig. 21G).

Development of muscle fibers

In newly hatched larvae of 2.14 mm mean TL, there were 5–6 layers of the developing white fibers containing myofibrils. Superficially a single layer of elliptical cells overlay the white fiber zone. These cells seemed to be presumptive myoblasts of the superficial red fibers, but no myofibril was contained within the cells (Fig. 22A).

In 21 hours old larvae of 2.90 mm mean TL, the myofibrils in the white fibers considerably increased in number. The superficial cells were clearly characterized in having a few myofibrils (Fig. 22B). Histochemical differences between the super-

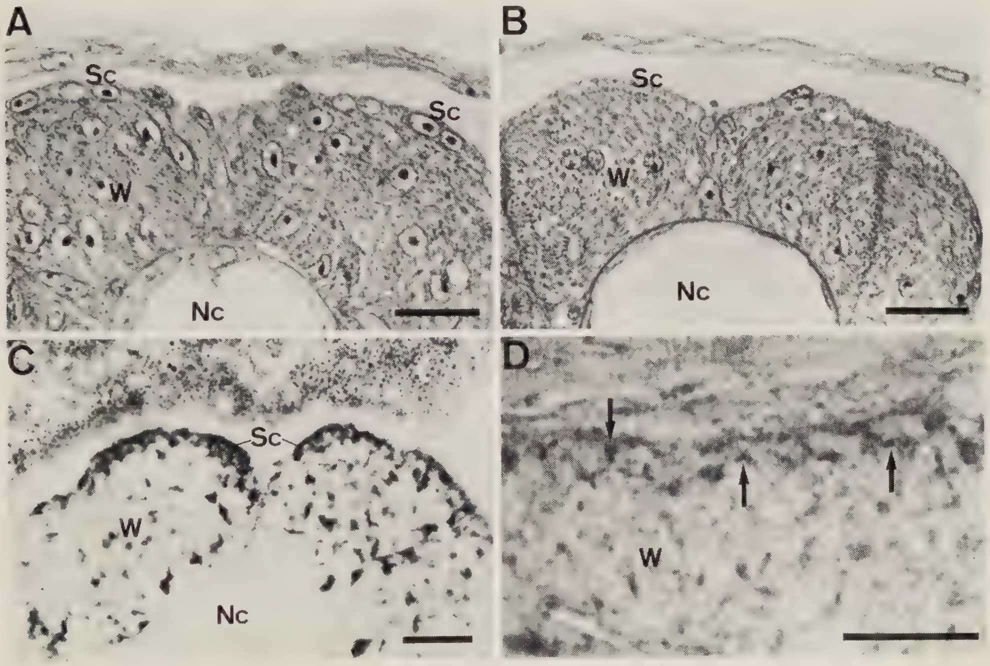


Fig. 22. Photomicrographs of myotomal muscle fibers in larvae of *Pagrus major*. A: transverse section of newly hatched larva, 2.14 mm TL, showing a single layer of superficial cells without myofibril and developing white fibers with myofibrils. B: transverse section of 2.90 mm TL larva showing a single layer of superficial cells with myofibrils and white fibers with a number of myofibrils. C: transverse section of 3.25 mm TL larva stained for SDH. Note intense staining of a single layer of superficial cells. D: transverse section of 3.25 mm TL larva stained for lipid. Note many lipid droplets (arrows) in superficial cell layer. Nc, notochord; Sc, superficial cells; W, white fibers. Scale bars indicate 20 μm.

ficial cells and inner white fibers were not recognized.

In 34 hours old larvae of 3.06 mm mean TL, the superficial cells showed more or less intense staining for SDH and NADHD as compared to the white fibers.

In 64.5 hours old larvae of 3.28 mm mean TL, satisfactory development was recognized in the organ systems related to onset of feeding, for example, the mouth opening and retinal pigmentation. Most larvae floated with the head directed downwards, and actual feeding activity was not observed.

Histochemical characters of the superficial cells were distinguishable at this stage, that is, these cells stained intensely for SDH and NADHD (Fig. 22C) and lightly for lipid (Fig. 22D).

In 87 hours old larvae of 3.31 mm mean TL, at onset of feeding, histological and histochemical characters of the red fibers were nearly the same to those at the preceding stage. The red fibers stained lightly for SDH and NADHD in the anterior trunk region and intensely backward to the caudal region.

No essential changes occurred in the myotomal muscle until the larvae grew to 7 mm TL, 14–17 days after hatching (Fig. 23A). The white fibers were largest in diameter in the deeper layer around the notochord, and decreased in diameter from there outward. After onset of feeding, these fibers increased in number in dorso-ventral direction rather than horizontal direction. During the postlarval interval, the white and red fibers stained equally for PhR (Fig. 23B).

In larvae of about 7 mm TL, small fibers were formed beneath a single layer of the superficial red fibers near the horizontal septum, and began to stain lightly for SDH and NADHD. These fibers seemed to be immature pink fibers (Fig. 23C). Developing pink fibers gradually increased in number and stained more intensely for SDH and NADHD with growth.

The small white fibers were also formed around the notochord.

In addition, small diameter fibers were formed between a single layer of the superficial red fibers and the skin near the horizontal septum. These seemed to be newly formed red fibers (Fig. 23C). The superficial red fibers were 2 layers thick at the level of the epaxial and hypaxial posterior cones of the myotomes.

The fibers of the dorsal and anal fin muscles showed relatively intense staining for SDH and NADHD, especially those attached to the cartilaginous pterygiophores.

In an early juvenile of 11.2 mm TL, three main zones of muscle fibers were clearly recognized in the anal region. The red fibers were 3–4 layers thick and the pink fibers were 5–6 layers thick near the horizontal septum. Newly formed small red fibers showed intense staining for SDH and NADHD as well as large red fibers (Fig. 23D). The posterior end of the myotomal muscle was mainly composed of the superficial red fibers. The fibers of the caudal fin muscles showed relatively intense staining for NADHD (Fig. 23E). On the other hand, in the anterior trunk

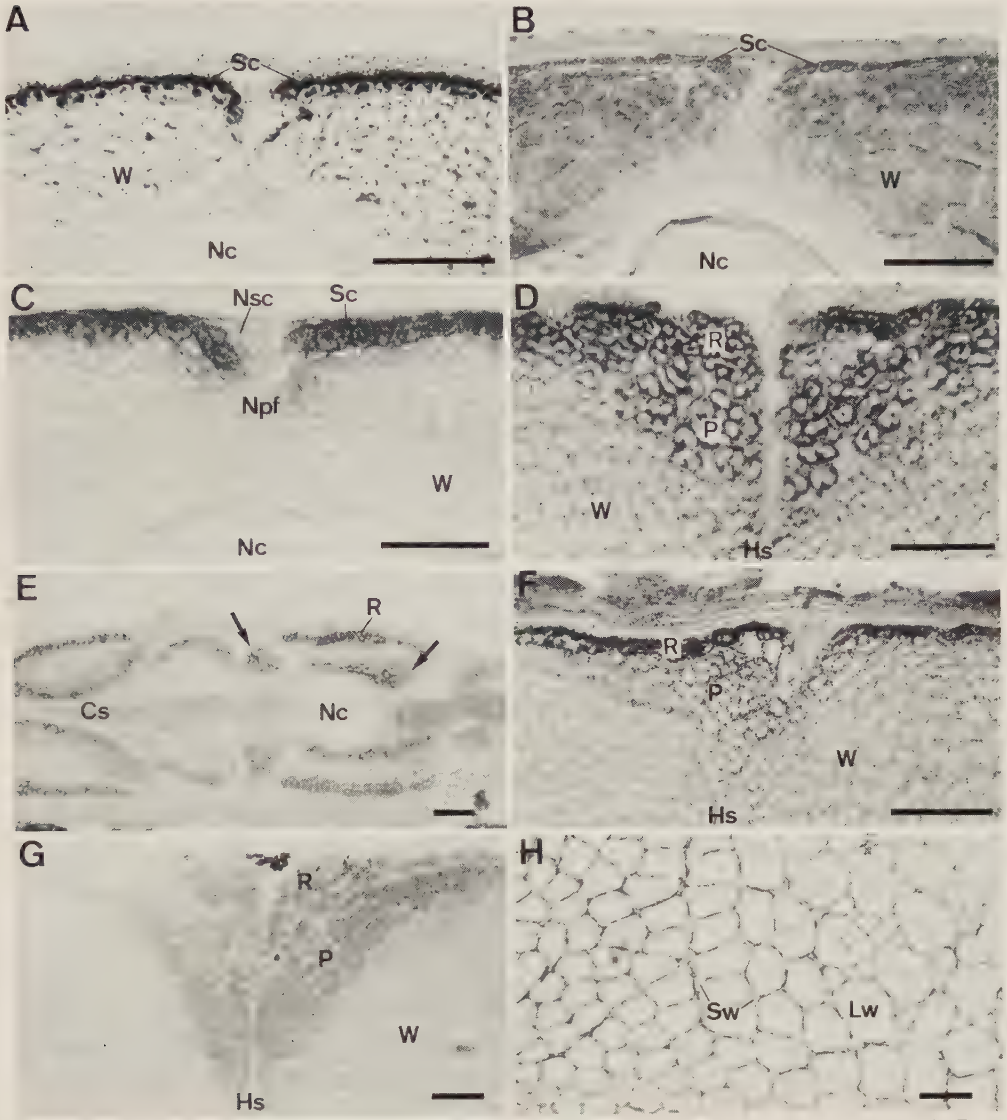


Fig. 23. Photomicrographs of myotomal muscle fibers in larvae and juveniles of *Pagrus major*. A: transverse section of 4.9 mm TL larva stained for NADHD. B: transverse section of 7.1 mm TL larva stained for PhR, showing similar staining of superficial cells and white fibers. C: transverse section of 8.0 mm TL larva stained for SDH. Note newly formed pink fibers and stratification of superficial cells. D: transverse section in anal region of 11.2 mm TL juvenile stained for NADHD. E: transverse section of caudal peduncle of 11.2 mm TL juvenile stained for NADHD. Note posterior end of myotomal musculature mainly composed of red fibers and intensely stained fibers (arrows) of caudal fin muscles. F: transverse section in anterior trunk region of 11.2 mm TL juvenile stained for NADHD. Note a single layer of red fibers and stratification of pink fibers. G: transverse section of 20 mm TL juvenile stained for PhR. H: transverse section of 20 mm TL juvenile stained for NADHD, showing newly formed small white fibers scattered among large white fibers. Cs, caudal skeleton; Hs, horizontal septum; Lw, large white fibers; Nc, notochord; Npf, newly formed pink fibers; Nsc, newly formed superficial cells; P, pink fibers; R, red fibers; Sc, superficial cells; Sw, small white fibers; W, white fibers. Scale bars indicate 50 μm.

region, the pink fibers were well developed, and the superficial red fibers were single layer thick (Fig. 23F).

The myotomal muscle in a juvenile of 20 mm TL was almost similar in structure to those of large wild specimens. The red and pink fibers stained for PhR, but the white fibers showed negative staining for PhR (Fig. 23G). The small white fibers partly occurred among the large white fibers of 40–50 μm in diameter, and transverse sections showed a mosaic appearance in this region as is the case with large wild specimen. These newly formed white fibers showed almost negative staining for SDH, NADHD and lipid, similar to large white fibers (Fig. 23H).

Discussion

Histochemical observations on three main fiber types of *P. major* are in general agreement with those reported by previous authors on the teleosts, except for PhR. The pattern of staining for PhR is in many respects similar to that given by CARPENÈ *et al.*,[78] but contrary to the results of PATTERSON *et al.*[79] that the white fibers stain more intensely for PhR than the red fibers.

Very small diameter fibers showing very weak or negative staining for SDH, NADHD, PhR and lipid are found to extend between the red and pink fiber zones in large wild specimens. Similar fibers are known to occur in some other teleosts.[78–81] PATTERSON *et al.*[79] suggested that these fibers represent the areas of continuing muscle growth. This hypothesis, however, may remain to be answered, because growing red and pink fibers scattered within each zone have respective histochemical characters even these are smaller in diameter than very small diameter fibers.

In many teleosts, the relative proportion of the red muscle increases toward the caudal region, and this is probably associated with greater flexure in the caudal region during swimming.[73,81,82] My results, that the red fibers are well developed in the caudal region, contrary to the pink fibers, may support this conception.

It is generally conceded that in the myotomal muscle of teleosts the red fibers differentiate after the formation of the deep white fiber zone. However, there exist considerable differences in the muscle development of teleost larvae at the time of hatching. In the cyprinid, *Brachydanio rerio*, a single layer of the superficial muscle cells with large myofibrils appears prior to hatching, in 30-somite[83] or 33-somite[84] embryos, and the first differences in metabolic activities between the deep and superficial cell populations occur in larvae, 7 days after fertilization.[83] In the brown trout, *Salmo trutta*, the differentiation of the superficial cells can be recognized prior to hatching.[85] In the rainbow trout, *Salmo gairdneri*, NAG and NURSALL[86] reported that the superficial red fibers are not distinguishable at the time of hatch-

ing. According to their description, however, the superficial cells at this stage contain lipid droplets and a number of mitochondria which are characteristics of the red fibers as pointed out by PROCTOR *et al.*[85] On the other hand, in larvae from pelagic eggs such as *P. major*, the superficial cells with myofibrils are first seen in 21 hours old larvae, and the metabolic properties of these cells are recognized prior to onset of feeding. According to O'CONNELL,[87] in the northern anchovy, *Engraulis mordax*, there are only two layers of oblique muscle fibers, which are peculiar to the clupeoids, at the time of hatching. The addition of new white fibers over the surface of the oblique muscle starts in larvae of 3.5-4.0 mm in standard length, at onset of feeding, and a single layer of the superficial red fibers appears at 6 or 7 mm SL.

In general, the pink fibers develop at later stages as compared to the white and red fibers. In *B. rerio*, the pink fibers are present by 2.5 months old.[84] In *S. trutta*, the pink fibers are observed in early alevins, and a marked increase of the superficial red fibers in the lateral line region is demonstrated at nearly the same time.[85] In *E. mordax*, a small pink fiber zone becomes evident and a second layer of the red fibers appears at about 14 mm SL.[87] My results on *P. major* indicated that the differentiation of the pink fibers and stratification of the superficial red fibers occur simultaneously at about 7 mm TL.

Some considerations have been made on the relationship between the differentiation of the red fibers and sustained swimming ability in larvae. According to FUKUHARA and KISHIDA,[21] larvae of *P. major* spend short period for motion and relatively long period for rest during the prelarval interval, but the drastic increase in swimming ability is evident at the time of yolk absorption. The results shown here that the superficial red fibers arranged in a single layer aquire the metabolic features shortly before yolk absorption, especially in the caudal region, suggest that the red fibers serve in these larvae to permit further feeding activity, as observed in *B. rerio*.[83] *P. major* larvae at this stage swim by active undulation of the caudal region and fanning of the pectoral fins for several seconds, following by motionless resting for a moment. The duration of active period becomes longer and longer as the larvae grow. FUKUHARA and KISHIDA[21] showed that the swimming activity increases remarkably in *P. major* larvae beyond 20 days after hatching (about 6 mm SL). This stage corresponds to the differentiation of the pink fibers and stratification of the red fibers. The myotomal muscle may enable the larvae to increase sustained swimming ability with aid of the fins, at least beyond this stage. The myotomal muscle in a 11.2 mm TL specimen is composed of three types of the muscle fiber zones, especially in the anal region, and that of a 20 mm TL specimen is not different from those of 68.4 and 103.8 mm TL specimens, also in the trunk region. It indicates that the myotomal muscle of *P. major* almost reaches adult

condition in structure and function in early juveniles.

1-2. Fiber Morphometry

In mammal, the number of the myotomal muscle fibers is determined by shortly after birth.[88] Even in shark, the postnatal increase of the white muscle fibers is not observed although the number of the red muscle fibers showed a slight increase.[89] Contrary to these, it is interesting that the number of the myotomal muscle fibers in teleostean fishes increases almost throughout life[90-92] or up to at least a limited size.[93] But there have been very few studied on the early growth in size and number of the myotomal muscle fibers in teleosts.

In addition to the qualitative information like the fiber type differentiation which was clarified in the preceding division, the quantitative information as a propulsive muscle mass is required. This division was devoted to pursue the morphometric growth dynamics of the three main fiber types (white, red and pink) of the myotomal muscle in larvae and juveniles of *P. major*.

Most parts of this division have already been presented by MATSUOKA.[94]

Materials and methods

Specimens examined were through laboratory-produced series from newly hatched larvae to juveniles up to 20.4 mm TL, reared in the Fisheries Research Station, Kyoto University in 1982. Incubation and rearing temperatures ranged from 20.4 to 25.0°C. These included some starved specimens at several stages, for 3-4 days, until one half of initial separated individuals died. Five larger specimens, ranged 23.4-28.8 mm TL, reared in Fukui Prefectural Fisheries Farming Center in 1980 were also used.

Total length of specimens fixed in 10% formalin solution was measured before embedding in methacryl resin. Transverse sections were cut at 2-3 μm around the 12th myomere from the posterior end of the opercle and stained with saturated solution of eosin in absolute alcohol.

Measurements were made on the fiber number and the cross-sectioned area on either right or left body side of the white, red and pink muscles, respectively. Three types of muscle fibers could be approximately identified, based on differences of distribution pattern of myofibrils within fiber.[85] The fiber number was directly counted with the use of binocular microscope. Outlines of the three muscle fiber zones were drawn on paper with the use of camera lucida. Then, these were cut out, weighed with microbalance and true areas were calculated. All values were doubled to give the total fiber number and the total cross-sectioned area on both

Table 1. Relationships between morphometric measurements of myotomal muscle fibers and total length in *Pagrus major*, on unstarved specimens reared in the Fisheries Research Station, Kyoto University. log *a* and *b* are constants in equation, log y=log $a+b$ log x. *r* shows correlation coefficient.

		stage	log *a*	*b*	*r*
Total number of fibers					
	White	<3.06 mm	2.258	0.098	0.1920
		3.06 mm−7.58 mm	0.555	3.603	0.9869
		>7.58 mm	3.594	0.148	0.0004
	Red	<3.10 mm	1.428	0.762	0.8481
		3.10 mm−6.11 mm	1.076	1.480	0.9741
		>6.11 mm	1.439	1.017	0.9833
	Pink	<9.70 mm	−1.215	3.677	0.9404
		>9.70 mm	1.549	0.876	0.8324
Total cross-sectioned area					
	White	<3.16 mm	3.291	0.846	0.5426
		3.16 mm−9.00 mm	1.705	4.014	0.9923
		>9.00 mm	3.052	2.602	0.9714
	Red	<2.92 mm	2.448	0.469	0.4722
		2.92 mm−8.28 mm	1.124	3.308	0.9890
		>8.28 mm	2.606	1.694	0.9746
	Pink	<9.77 mm	−3.848	7.915	0.9800
		<9.77 mm	1.240	2.774	0.9448
Average fiber area					
	White	<4.82 mm	1.142	0.453	0.5418
		4.82 mm−6.20 mm	1.695	−0.355	−0.7205
		>6.20 mm	−0.510	2.427	0.9674
	Red	<2.86 mm	1.053	−0.382	−0.4926
		2.86 mm−8.51 mm	0.017	1.884	0.9517
		>8.51 mm	1.037	0.786	0.8652
	Pink	<9.59 mm	−2.612	4.229	0.9663
		>9.59 mm	−0.385	1.961	0.8752

body sides. The average fiber area was derived from the total cross-sectioned area devided by the total fiber number.

Results

The total number of fibers, the total cross-sectioned area and the average fiber area were plotted against total length on log/log co-ordinates (Figs. 24, 25, 26). The results of analyses by least squares regression of the allometry equation ($y=ax^b$ or log y=log $a+b$ log x, where y=morphometric measurements of fibers, x= total length, a and b=constantus) calclated on unstarved specimens reared in the

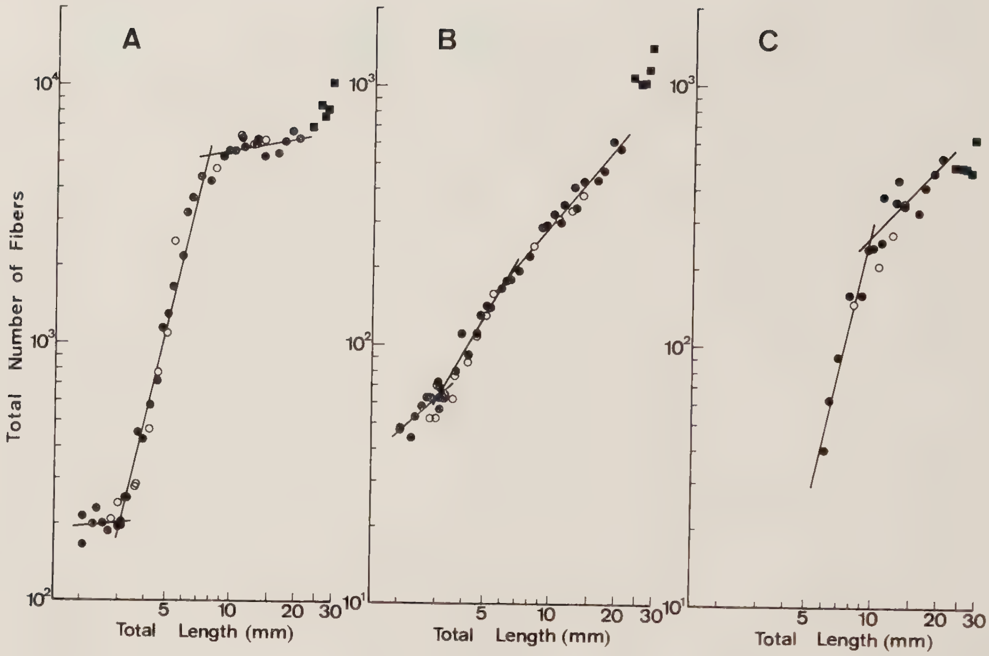


Fig. 24. Logarithmic plots showing relationship between total number of fibers and total length in *Pagrus major*. Regression lines are drawn for specimens indicated by solid circles. A: white fibers. B: red fibers. C: pink fibers. ●, unstarved specimens reared in the Fisheries Research Station, Kyoto University; ○, starved specimens from same source; ■, specimens reared in Fukui Prefectural Farming Center.

Fisheries Research Station, Kyoto University (solid circle in Figs. 24, 25 and 26) were summarized in Table 1.

Total number of fibers

The total number of the white fibers (Fig. 24A) was almost constant, about 200, during the prelarval interval (2–3 mm TL). Shortly after onset of feeding, it increased remarkably (regression coefficient=b: 3.603). It reached about 5300 at 7.58 mm TL (calculated inflection point) and later shifted to a very slight increase (b: 0.148) up to about 20 mm TL. Some larger specimens (solid squares in Fig. 24A) showed a second rapid increase.

In the red fibrs (Fig. 24B), the total number increased gradually from 45 to 65 during the prelarval interval (b: 0.762) in contrast to the white fibers. A relatively rapid increase (b: 1.428) occurred between 3.10 and 6.11 mm TL. Later, the regression coefficient decreased (b: 1.017) but the total number reached 580 at 20 mm TL.

The newly formed pink fibers were first observed at about 6 mm TL and the total number of the pink fibers increased rapidy (b: 3.677) from the first appear-

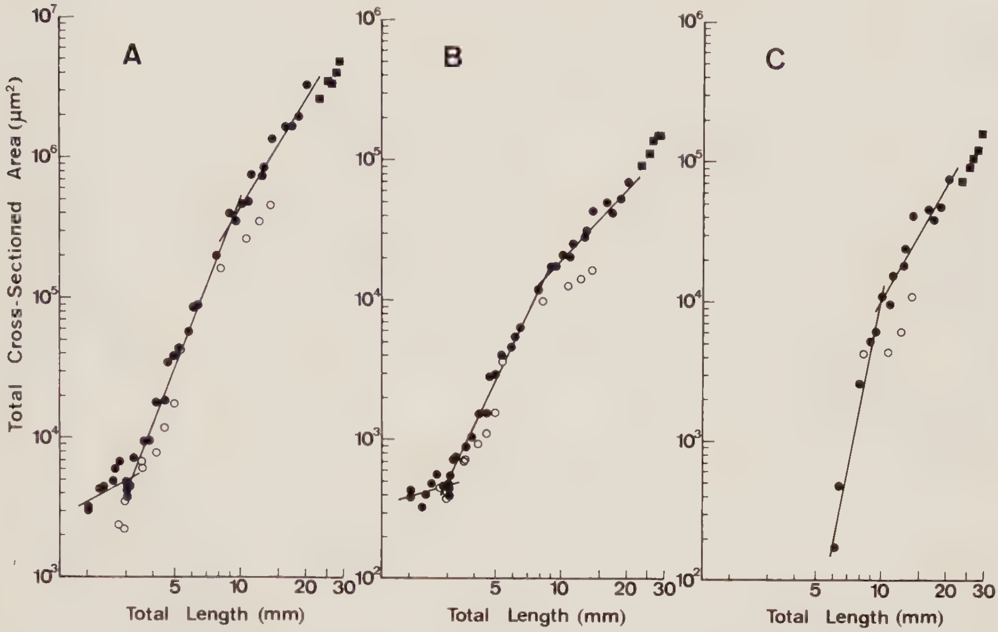


Fig. 25. Logarithmic plots showing relationship between total cross-sectioned area and total length in *Pagrus major*. A: white fibers. B: red fibers. C: pink fibers. Symbols are the same in Fig. 24.

ance (Fig. 24C) in contrast to the white and red fibers. Later, the regression coefficient certainly decreased beyond 9.70 mm TL (*b* 0.876) but the total number reached 490 at 20 mm TL.

No significant difference was deteced between unstarved and starved specimens (Fig. 24).

Total cross-sectioned area

The total cross-sectioned area of the white fibers (Fig. 25A) increased gradually during the prelarval interval (*b*: 0.846). A very rapid increase occurred between 3.16 and 9.00 mm TL (*b*: 4.014). Later, the regression coefficient decreased (*b*: 2.602) but the total cross-sectioned area reached 2700,000 μm^2 at 20 mm TL.

In the red fibers (Fig. 25B), the total cross-sectioned area showed a slight increase during the prelarval interval (*b*: 0.469) and very rapid increase occurred between 2.92 and 8.28 mm TL (*b*: 3.308). Later, the regression coefficient decreased (*b*: 1.694) but the total cross-sectioned area reached 65,000 μm^2 at 20 mm TL.

The total cross-sectioned area of the pink fibers (Fig. 25C) increased rapidly from the first appearance (*b*: 7.915). Later, (beyond 9.77 mm TL), the regression coefficent decreased (*b*: 2.774) but the total cross-sectioned area reached 71,000 μm^2 at 20 mm TL.

Starved specimens had only about one half of the total cross-sectioned area of

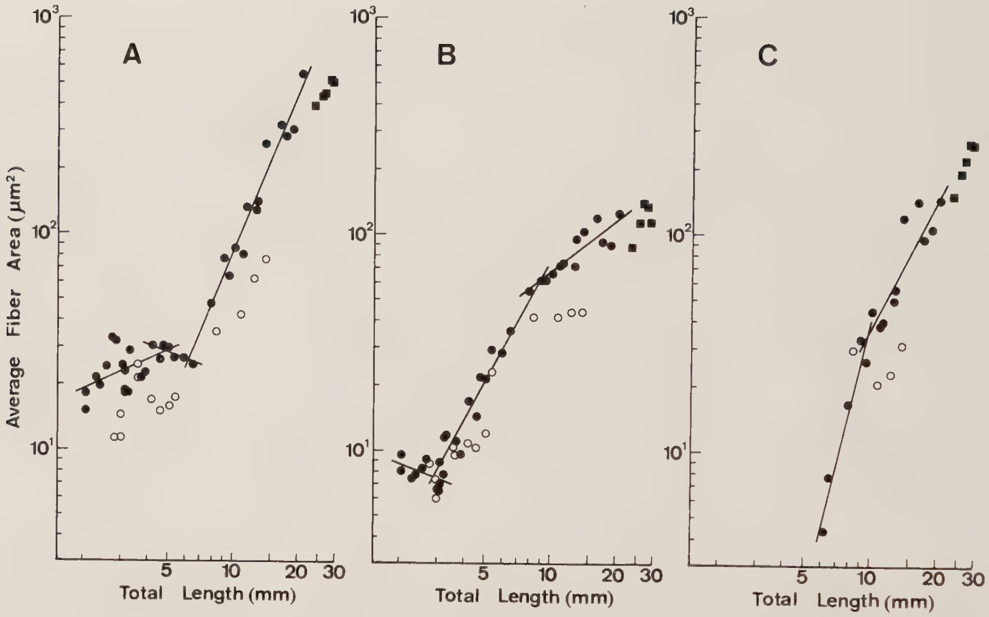


Fig. 26. Logarithmic plots showing relationship between average fiber area and total length in *Pagrus major*. A: white fibers. B: red fibers. C: pink fibers. Symbols are the same in Fig. 24.

the white fibers at onset of feeding and the notable decreased area in almost all specimens beyond this stage, as compared with unstarved specimens. The total cross-sectioned area of the red and pink fibers in starved specimens also showed notable decreases except at onset of feeding in the former (Fig. 25).

Average fiber area

The average fiber area of the white fibers (Fig. 26A) increased slightly from hatching up to about 30 μm^2 (b: 0.453) and showed a little decrease between 4.82 and 6.20 mm TL (b: −0.355) in spite of a somewhat wide distribution of plots in early stages. Later, it shifted to a rapid increase (b: 2.427) and reached 440 μm^2 at 20 mm TL.

In the red fibers (Fig. 26B), the average fiber area showed a significant decrease during the prelarval interval (b: −0.384) and a rapid increase occurred between 2.86 and 8.51 mm TL (b: 1.884). Later, the regression coefficient decreased (b: 0.786) but the average fiber area reached 120 μm^2 at 20 mm TL.

The average fiber area of the pink fibers (Fig. 26C) increased rapidly from the first appearance (b: 4.229). Later (beyond 9.59 mm TL), the regression coefficient decreased (b: 1.961) but the average fiber area reached 150 μm^2 at 20 mm TL.

As compared with unstarved specimens, the average fiber area of three types of the muscles of starved specimens showed notable decreases as in the case of

the total cross-sectioned area.

Discussion

Plots of morphometric measurements of the myotomal muscle fibers in the early growth of *P. major* indicate the existence of some stanzas (term adopted from MARTIN[95]) delimited by inflections. The first inflections of morphometric measurements of the white and red fibers except the average fiber area of the white fibers are concentrated to the transitional stage from prelarva to postlarva, about 3 mm TL measured in fixed specimens. It is also observed that metabolic properties of the red fibers appear shortly prior to onset of feeding, shown in the preceding division. These facts show that the qualitative and quantitative changes of the myotomal muscle fibers occur around this stage.

The most considerable change of fiber measurements between the prelarval and postlarval intervals is that of the total number of the white fibers. The rapid increase of the total number of the white fibers occurs only after exogenous food uptake, because starved specimens for 3.5 days after yolk absorption show no increase of the white fiber number. During starvation, the total cross-sectioned area and the average fiber area of the white fibers significantly decrease but those of the red fibers scarcely decrease. It may indicate a preferential utilization of the white muscle during starvation,[96] although, in later stages, the total cross-sectioned area and the average fiber area of both white and red fibers decrease during starvation.

The rapid increase of the total number of the white fibers after onset of feeding is due to addition of small fibers in dorso-ventral direction rather than horizontal direction as shown in the preceding division. A slight decrease of the average fiber area of the white fibers between 4.82 and 6.20 mm TL indicates the occurrence of numerous small fibers, although each existing fiber is hypertrophying.

It is clarified in the preceding division that the differentiation of the pink fibers and the stratification of the red fibers which are the most significant qualitative changes in the postlarval interval occur in living specimens of *P. major* measuring about 7 mm TL. The appearance of small pink fibers in specimens of about 6 mm TL observed here is not contradictory to the former results, thinking of about 10% shrinkage in total length by fixiation in this stage. Following those changes, all second inflections of morphometric measurements of the white and red fibers and inflections of the pink fibers occur successively between about 6 and 10 mm TL, transitional interval to juvenile (postlarval stage II of KITAJIMA[13]). The considerable increase of swimming activity during this interval[21] must be accomplished by the muscle fiber growth in size and number including the white fibers as well

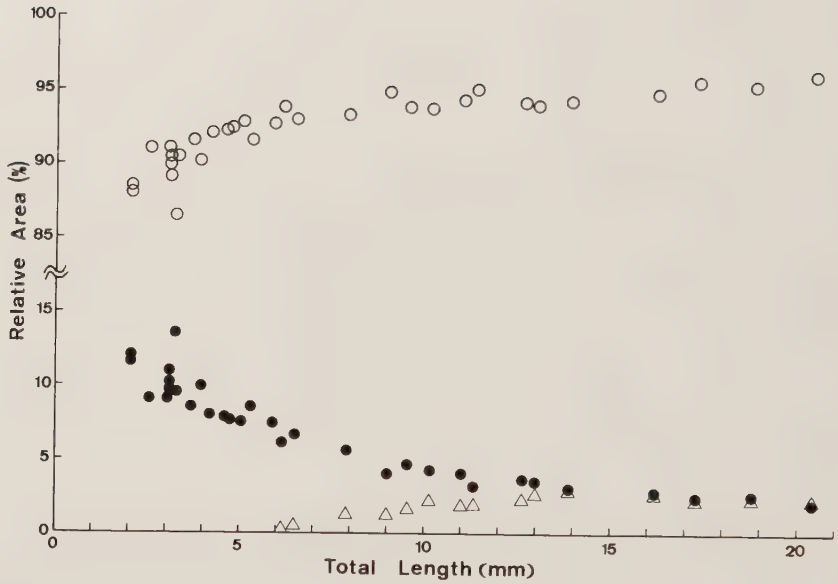


Fig. 27. Plots showing relationship between relative area (%) of three muscles and total length in *Pagrus major*. ○, white muscle; ●, red muscle; △, pink muscle.

as the differentiation of the pink fibers and the stratification of the red fibers. For example, the white fibers at 10 mm TL are about 30 times in number and about 90 times in the total cross-sectioned area against those of 3 mm TL.

Primarily, it was thought that the relative proportion of the red muscle within the myotomal muscle would increase with augment of sustained slow swimming ability. Figure 27 shows, however, a significant decrease of relative area of the red muscle with growth from about 10% to 2.5%, contrary to the increase in the white muscle from about 90% to 95%. A similar decrease of the red muscle ratio with growth is also observed in shark.[89] In regard of this point, further detailed investigations, for example, qualitative information of myofibrils in respective muscle types, would be required.

There are two alternative suggestions concerning the proliferation of muscle fibers: these are the splitting of existing fibers and new fiber formation from myosatellites among existing fibers. In the white fibers, evidence is accumulating that new fibers are differentiated from myosatellites,[91,97] although RAAMSDONK *et al.*[98] suggested the splitting in the white fibers. The white muscle of *P. major* begins to show a mosaic appearance containing both large and small fibers by 20 mm TL as shown in the preceding division. These developing small fibers are considered to be differentiated from myosatellites between existing large fibers. The second rapid increase in the number of the white fibers shown here (Fig. 24A) is mainly due to

the formation of new fibers among existing fibres. DAVISON[99] did not observe a mosaic appearance of the white fibers in the bully, *Gobiomorphus cotidianus*, even in the maximum length examined (about 10 cm). In the Mugilidae, small white fibers begin to be formed among large fibers at the beginning of September on 2 years old.[97] These observations seem to show that there are considerable differences in fish size at the beginning of new fiber formation among existing white fibers.

It is indicated in the northern anchovy, *Engraulis mordax*, that the red fibers may proliferate by equal vertical division.[87] In the brown trout, *Salmo trutta*, PROCTOR *et al.*[85] could not decide whether the origin of small red fibers appeared between the superficial mono-layer of existing red fibers and the skin around the lateral line region is due to splitting or not, because these fibers are ultrastructurally at the same developmental level as the adjacent large red fibers but have less advanced metabolic properties. Similar observations were also made in *P. major*. During the prelarval interval of *P. major*, the average fiber area of the superficial red fibers arranged in a single layer decreased as the result that the total fiber number significantly increases but the total cross-sectioned area increases slightly. This fact may support the splitting hypothesis of the red fibers, at least in early stages of *P. major*.

2. Head and Fin Muscles

There has been a great deal of literature concerning the head muscles of adult fishes. For example, a comprehensive description in teleosts,[100] detailed descriptions by serial reconstruction method in *Gasterosteus aculeatus*[101] and *Haplochromis elegans*,[102] etc. The head muscles have important functions in feeding and respiration by opening and closing the mouth and the buccal and branchial cavities. However, few literature has been presented about the development of the head muscles,[103–107] probably because of technical difficulty and comlexity of observation.

On the fin muscles of adult fishes, WINTERBOTTOM[100] also gave a comprehensive description. NAG[108] reported the caudal structure, including the caudal fin muscles, in the Clupeiformes and Perciformes, and VIDELER[109] showed the caudal fin muscles of *Tilapia nilotica*. Although the fin muscles have several important functions in swimming, there has been no information concerning the development of the fin muscles in teleosts.

This section was devoted to pursue the developmental process of the head muscles, mainly the jaw adductor muscle and its surrounding muscles, and the pectoral and caudal fin muscles of *P. major*, using graphical reconstruction technique of serial histological sections.

Materials and methods

Main specimens examined were laboratory-produced series from newly hatched larvae to juveniles up to 18.8 mm TL, reared in the Fisheries Research Station, Kyoto University in 1982. Incubation and rearing temperatures ranged from 20.4 to 25.0℃. Supplemental observations of the caudal fin muscles were made on several specimens reared in the Fisheries Research Station, Kyoto University in 1979 and Fukui Prefectural Fisheries Farming Center in 1980.

Total length of specimens fixed in Bouin's solution was measured before embedding in paraffin wax. Specimens beyond 15 mm TL were decalcified in 5% trichloroacetic acid solution for four days, before embedding. Serial transverse sections were cut at 6 or 8 μm, depending on fish size, and stained with Mallory-Heidenhain staining procedure or its modification (azocarmine G replaced by Mayer's hematoxylin). Better contrast of muscular tissues was obtained by the modified staining procedure.

Observations were made under a binocular microscope. Graphical reconstructions of the peripheral head muscles (not including the branchial and eye muscles) and the pectoral fin muscles were made from the photomicrographs of serial sections of seven specimens (2.95–11.3 mm TL). Reconstructions of the caudal fin muscles were carried out on two specimens of 6.00 and 11.3 mm TL.

In addition to reared larvae and juveniles, two large wild specimens, 106.2 and 110.5 mm TL, were used for dissection of the head and pectoral and caudal fin muscles under a stereomicroscope with camera lucida. Those specimens were caught in Maizuru Bay (Kyoto Prefecture) and Obama Bay (Fukui Prefecture) in 1981, respectively, and preserved in 10% formalin solution until dissection.

Muscular terminology generally followed WINTERBOTTOM.[100]

Results

Figure 28 shows the dissected peripheral head muscles, especially the jaw adductor muscle, of a 110.5 mm TL *P. major*. The adductor mandibulae consisted of four sections, A1, A2, A3 and Aw (Aw is not shown in the figure). A2 was the largest section which was located most peripherally and occupied the large part of the cheek. A1 was indistinctly subdivided into two parts, anterior and posterior ones. The anterior part was attached to the maxillary through a slender but strong tendon. A3 was located on the medial side of A2 and inserted on the broad tendon which was ultimately attached to the dentary. The levator arcus palatini lying at the rear of the orbit was a relatively small muscle. Around the opercular region, there were the levator pectoralis, dilatator operculi, levator operculi and ad-

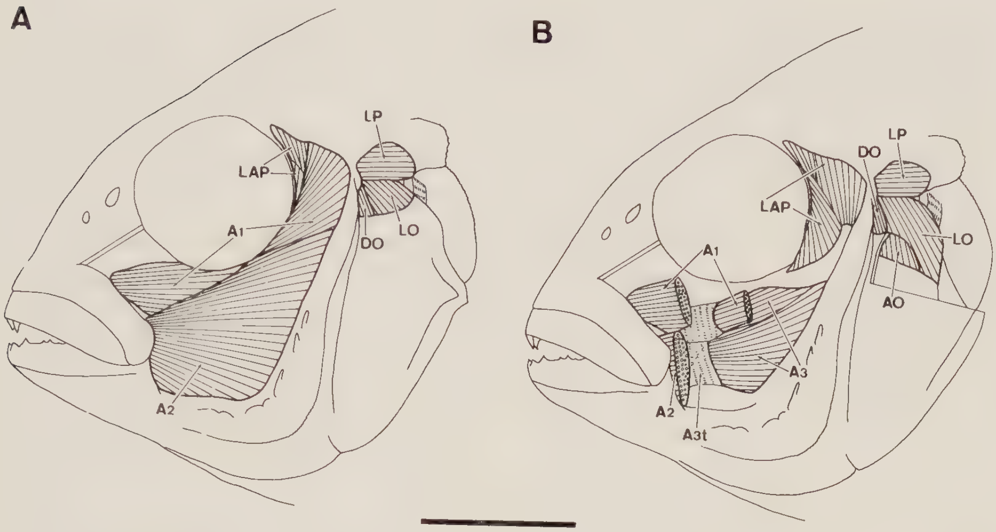


Fig. 28. Peripheral head muscles in *Pagrus major*, 110.5 mm TL (dissection). A: skin is removed. B: posterior regions of adductor mandibulae A1 and A2 and upper region of opercle are removed. Lateral view. A1, adductor mandibulae A1; A2, adductor mandibulae A2; A3, adductor mandibulae A3; A3t, tendon of adductor mandibulae A3; AO, adductor operculi; DO, dilatator operculi; LAP, levator arcus palatini; LO, levator operculi; LP, levator pectoralis. Scale bar indicates 10 mm.

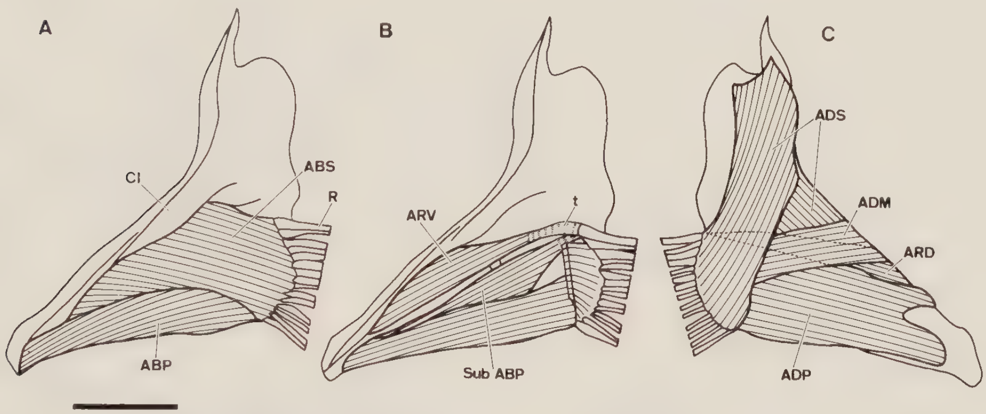


Fig. 29. Pectoral fin muscles in *Pagrus major*, 106.2 mm TL (dissection). A: lateral view, skin is removed. B: lateral view, abductor superficialis is removed. C: medial view. ABP, abductor profundus; ABS, abductor superficialis; ADM, adductor medialis; ADP, adductor profundus; ADS, adductor superficialis; ARD, arrector dorsalis; ARV, arrector ventralis; Cl, cleithrum; R, soft ray; Sub ABP, subdivision of abductor profundus; t, tendon. Scale bar indicates 5 mm.

ductor operculi. The latter three muscles were directly attched to the opercle.

Figure 29 shows the dissected pectoral fin muscles of a 106.2 mm TL specimen. The lateral pectoral muscles consisted of three components, the abductor superficialis, arrector ventralis and abductor profundus. The arrector ventralis served the marginal (first) ray. The abductor profundus was subdivided into the upper and lower fibers (Fig. 29A, B). The medial pectoral fin muscles consisted of five components, the adductor superficialis, arrector dorsalis, adductor medialis, adductor profundus and adductor radialis. The adductor radialis is not shown in the figure, but lied on the medial side of the actinosts (Fig. 29C).

The developmental process of the head and pectoral fin muscles is described

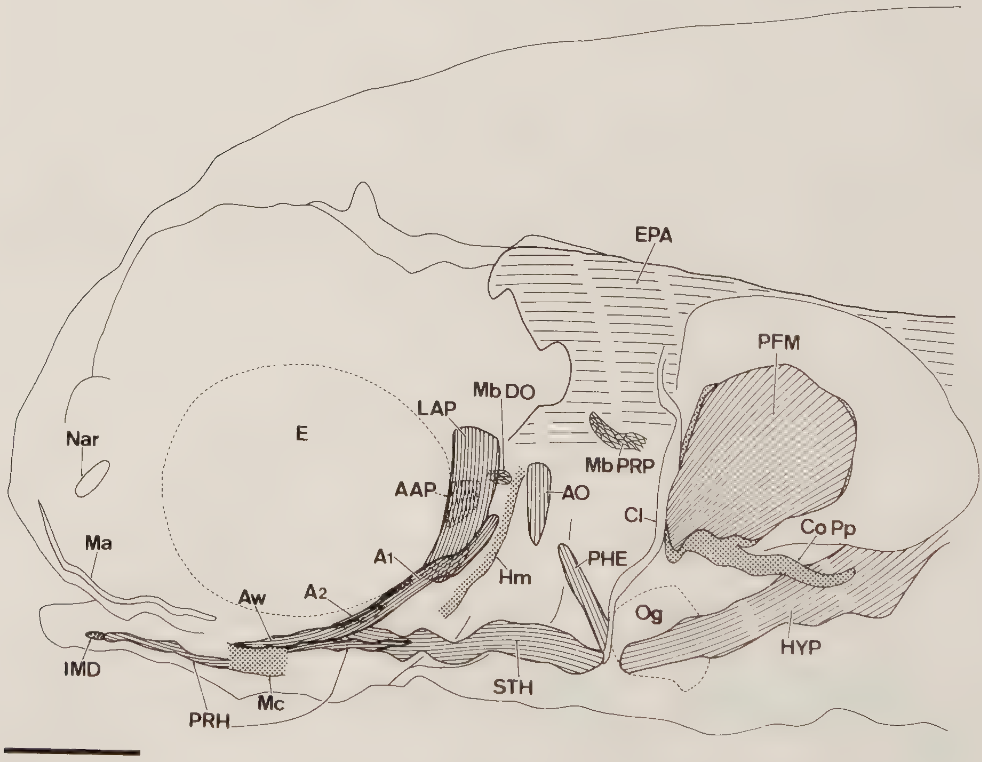


Fig. 30. Reconstruction of head and pectoral fin muscles in *Pagrus major*, 2.95 mm TL. Lateral view. Cartilaginous and bony elements are partly shown. A1, adductor mandibulae A1; A2, adductor mandibulae A2; AAP, adductor arcus palatini; AO, adductor operculi; Aw, adductor mandibulae Aw; Cl, cleithrum; Co Pp, coracoid posterior process; E, eye; EPA, epaxialis; Hm, hyomandibular; HYP, hypaxialis; IMD, intermandibularis; LAP, levator arcus palatini; Ma, maxillary; Mb DO, myoblast of dilatator operculi; Mb PRP, myoblast of protractor pectoralis; Mc, Meckel's cartilage; Nar, naris; Og, oil globule; PFM, pectoral fin muscle; PHE, pharyngoclavicularis externus; PRH, protractor hyoidei; STH, sternohyoideus. Scale bar indicates 0.1 mm.

as follows. In a 2.75 mm larva, 38.5 hours old from hatching, no fiber with myofibrils was discerned in the head and pectoral fin regions except for the myotomal muscle. The head and pectoral fin muscles were partly at the state of myoblast.

In a 63 hours old larva of 2.95 mm TL, shortly prior onset of feeding, the adductor mandibulae, levator arcus palatini, adductor arcus palatini, adductor operculi, intermandibularis, protractor hyoidei, sternohyoideus and pharyngoclavicularis externus were differentiated as muscle fiber masses with myofibrils. The adductor arcus palatini was situated posteriorly to the orbit. The levator arcus palatini was a relatively large muscle, compared with other muscles at this stage. The adductor mandibulae A1 and Aw were completely connected and formed a slender rod-like muscle. Aw was attached to the Mekel's cartilage. A2 was a small rod-like muscle lying on the dorsomedial side of A1. The dilatator operculi and protractor pectoralis were still at the state of myoblast. The pectoral fin muscles consisted of two flat muscles lying on the both sides of the fin plate cartilage (Fig. 30).

In a 3 days and 14 hours old larva of 2.95 mm TL, shortly after onset of feeding, the dilatator operculi and protractor pectoralis became muscle fiber masses

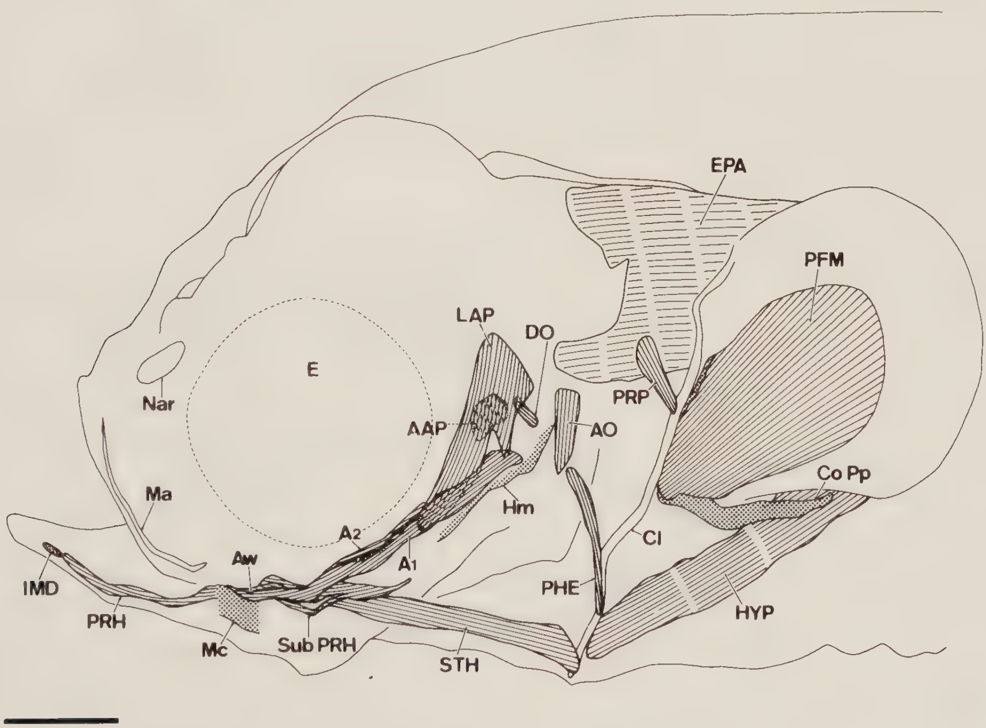


Fig. 31. Reconstruction of head and pectoral fin muscles in *Pagrus major*, 2.95 mm TL. Lateral view. Cartilaginous and bony elements are partly shown. DO, dilatator operculi ; PRP, protractor pectoralis ; Sub PRH, subdivision of protractor hyoidei. For other abbreviations, see Fig. 30. Scale bar indicates 0.1 mm.

with myofibrils. The subdivision of the protractor hyoidei was formed (Fig. 31).

In a 5 days and 17 hours old larva of 3.20 mm TL, the head muscles were almost similar to those of the preceding stage.

In a 9.5 days old larva of 4.40 mm TL, the levator operculi and pharyngoclavicularis internus were added to the head muscles. The formation of the adductor arcus palatini proceeded anteriorly. A slight constriction was observed between the adductor mandibulae A1 and Aw. A2 expanded ventrally and was tendinously attached to the Meckel's cartilage (Fig. 32).

In a 14.5 days old larva of 5.20 mm TL, the hyohyoidei inferioris, hyohyoidei adductores and hyohyoidei abductores were formed. The protractor hyoidei and sternohyoideus were considerably thickened. The constriction between the adductor mandibulae A1 and Aw was conspicuous and those were connected with only a few fibers. The tendon of A1 was partly recognizable, but it was attached to neither A1 nor the maxillary. The pectoral fin muscles, consisting of two flat muscles,

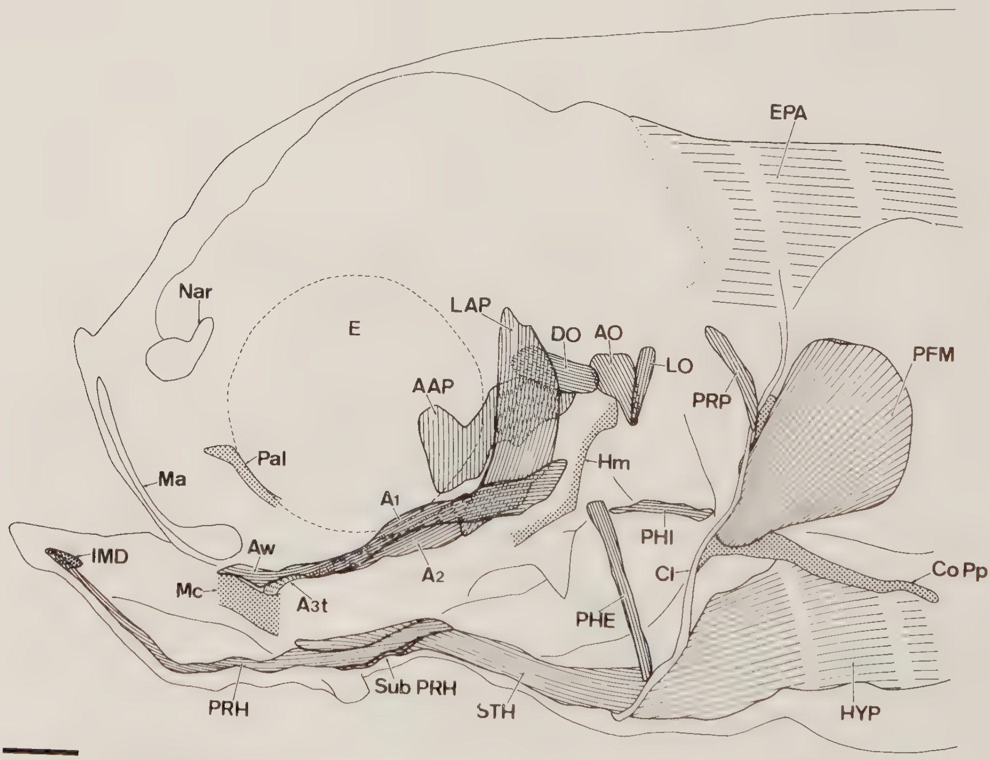


Fig. 32. Reconstruction of head and pectoral fin muscles in *Pagrus major*, 4.40 mm TL. Lateral view. Cartilaginous and bony elements are partly shown. A3t, tendon of adductor mandibulae A3; LO, levator operculi; Pal, palatine; PHI, pharyngoclavicularis internus. For other abbreviations, see Figs. 30 and 31. Scale bar indicates 0.1 mm.

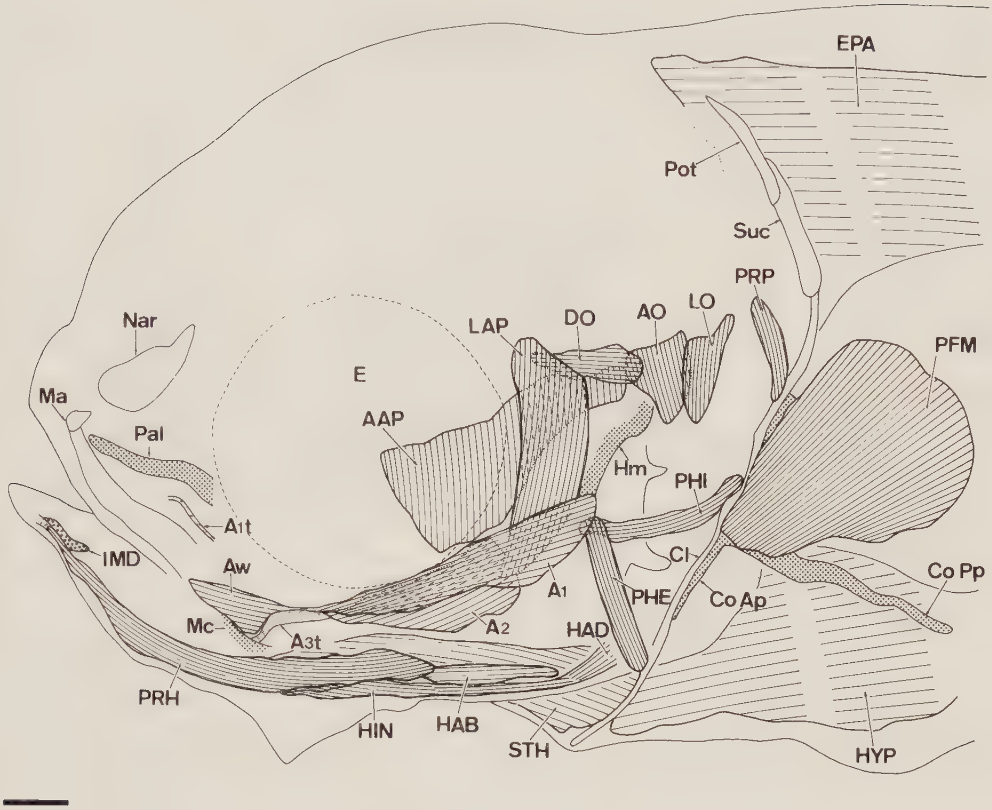


Fig. 33. Reconstruction of head and pectoral fin muscles in *Pagrus major*, 5.20 mm TL. Lateral view. Cartilaginous and bony elements and hyohyoidei adductores are partly shown. Alt, tendon of adductor mandibulae A1; Co Ap, coracoid anterior process; HAB, hyohyoidei abductores; HAD, hyohyoidei adductores; HIN, hyohyoidei inferioris; Pot, posttemporal; Suc, supracleithrum. For other abbreviations, see Figs. 30, 31 and 32. Scale bar indicates 0.1 mm.

were at the same state as the preceding stages (Fig. 33).

In a 18.5 days old larva of 6.80 mm TL, the adductor mandibulae A1 and Aw were completely separated. A1, A2 and Aw considerably expanded in dorso-ventral direction. The tendon of A1 was attached to the medial face of the maxillary. The pectoral fin muscles began to differentiate to its respective components. The anterior region of the lateral flat muscle was divided into two layers. The more lateral layer corresponded to the future abductor superficialis and the medial one was subdivided into the future arrector ventralis and abductor profundus. The anterior region of the medial flat muscle was also divided into two layers. The more medial layer corresponded to the future adductor superficialis and the lateral layer to the future arrector dorsalis and adductor profundus (Fig. 34).

In a 29.5 days old juvenile of 11.3 mm TL, all components of the peripheral

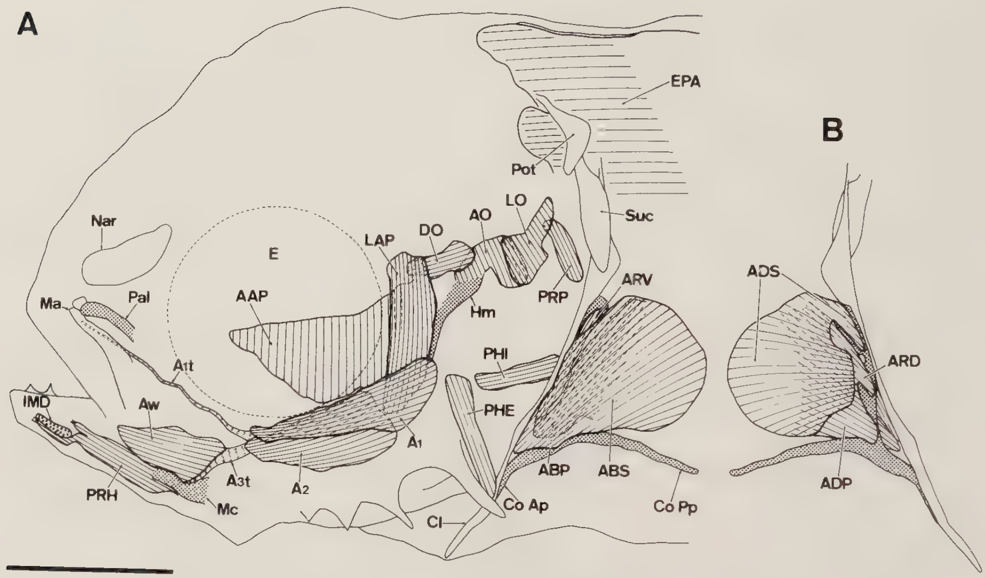


Fig. 34. Reconstruction of head and pectoral fin muscles in *Pagrus major*, 6.80 mm TL. A: lateral view. B: medial view of pectoral fin. Cartilaginous and bony elements and protractor hyoidei are partly shown. Hyohyoidei abductores, hyohyoidei adductores, hyohyoidei inferioris, hypaxialis, sternohyoideus and pectoral fin fold are not shown. ABP, abductor profundus; ABS, abductor superficialis; ADP, adductor profundus; ADS, adductor superficialis; ARD, arrector dorsalis; ARV, arrector ventralis. For other abbreviations, see Figs. 30, 31, 32 and 33. Scale bar indicates 0.5 mm.

head and pectoral fin muscles were discernible. Since the adductor mandibulae A3 could not be clearly distinguished from A2 as a separate muscle mass at this stage and also the preceding stages, both A2 and A3 was treated as A2 for convenience. Aw was secondarily connected with A2. A1, A2 and Aw became large and thickened, while the levator arcus palatini which had been a relatively large muscle at early stages showed no conspicuous growth in size. The formation of the adductor arcus palatini reached the anterior margin of the orbit. The levator pectoralis was attached to the upper part of the pectoral girdle. The pectoral fin muscles were almost similar to those of a 106.2 mm TL specimen. The arrector ventralis was completely separated from the abductor profundus and the subdivision of the abductor profundus was observed. The adductor medialis and adductor radialis were formed (Fig. 35).

Figure 36 shows the dissected caudal fin muscles of a 110.5 mm TL specimen. The caudal skeleton was entirely covered by the caudal fin muscles. These muscles consisted of seven components, the flexor dorsalis superior, flexor dorsalis, hypochordal longitudinalis, flexor ventralis, flexor ventralis externus, flexor ventralis in-

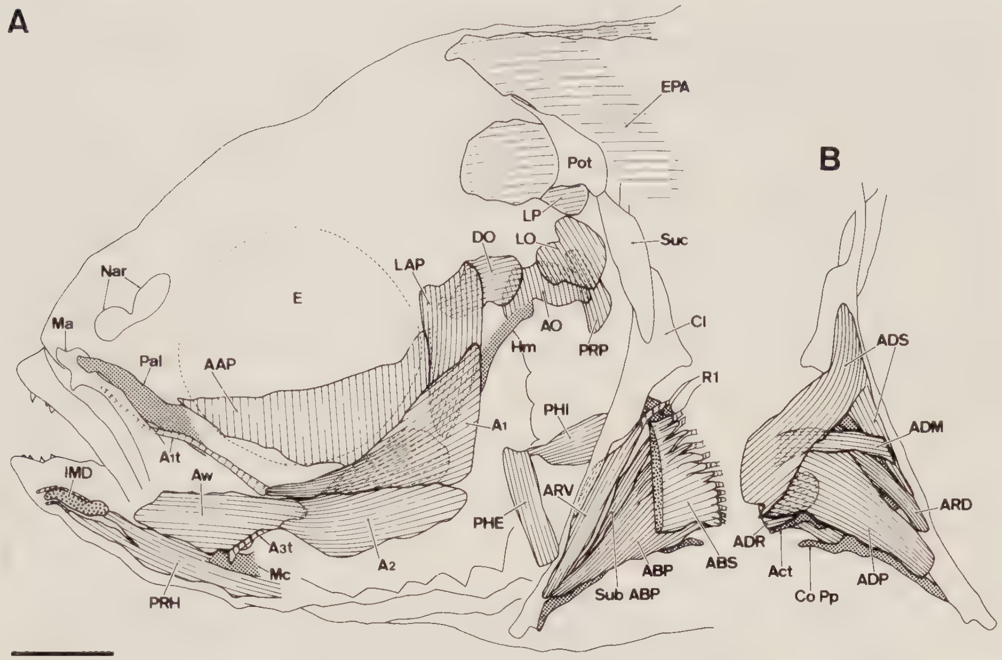


Fig. 35. Reconstruction of head and pectoral fin muscles in *Pagrus major*, 11.3 mm TL. A: lateral view. B: medial view of pectoral fin. Cartilaginous and bony elements, pectoral fin ray, protractor hyoidei and abductor superficialis are partly shown. Hyohyoidei abductores, hyohyoidei adductores, hyohyoidei inferioris, hypaxialis, sternohyoideus are not shown. Act, actinost; ADM, adductor medialis; ADR, adductor radialis; LP, levator pectoralis; R, soft ray; Sub ABP, subdivision of abductor profundus. For other abbreviations, see Figs. 30, 31, 32, 33 and 34. Scale bar indicates 0.5 mm.

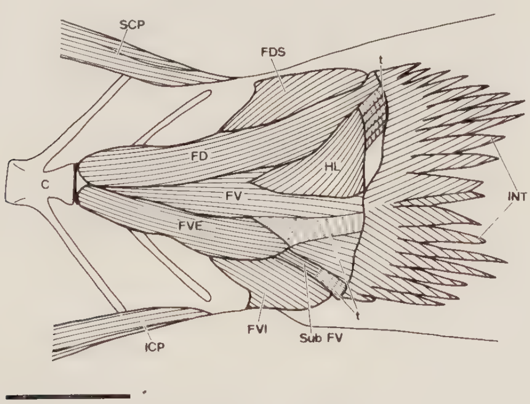


Fig. 36. Caudal fin muscles in *Pagrus major*, 110.5 mm TL (dissection). Lateral view. C, centrum; FD, flexor dorsalis; FDS, flexor dorsalis superior; FV, flexor ventralis; FVE, flexor ventralis externus; FVI, flexor ventralis inferior; HL, hypochordal longitudinalis; ICP, infracarinalis posterior; INT, interradialis; SCP, supracarinalis posterior; Sub FV, subdivision of flexor ventralis; t, tendon. Scale bar indicates 5 mm.

ferior and interradialis. The flexor ventralis contained a slender ventral subdivision. The hypochordal longitudinalis, flexor ventralis externus and subdivision of the flexor ventralis were tendinously attached to the caudal fin rays.

Unfortunately, I could not study further the detailed developmental process of the caudal fin muscles due to the limited number of completely serial setcions. I was able to use only two specimens for graphical reconstruction of the caudal region.

In a 13 days old larva of 4.60 mm TL, the caudal fin muscles were not formed yet.

In a 15.5 days old larva of 6.00 mm TL, all components of the caudal fin mus-

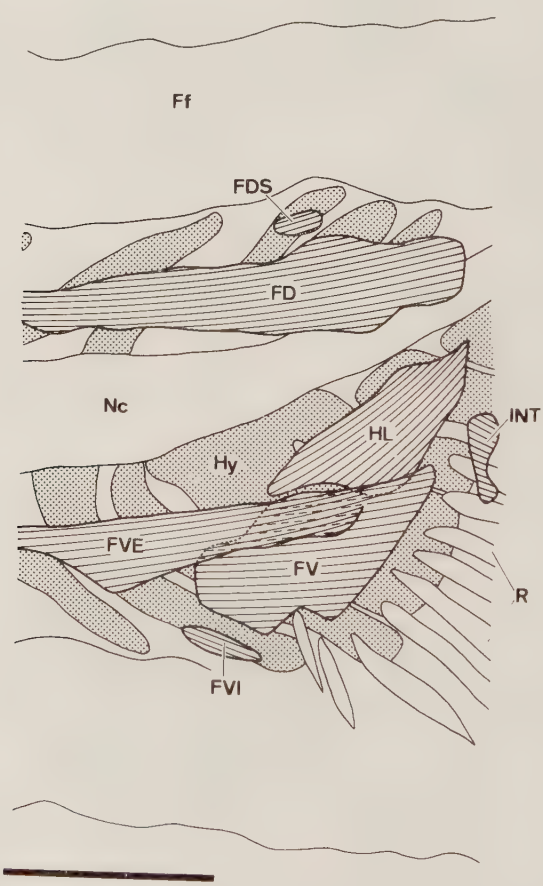


Fig. 37. Reconstruction of caudal muscles in *Pagrus major*, 6.00 mm TL. Lateral view. Cartilaginous elements are partly shown. FD, flexor dorsalis; FDS, flexor dorsalis superior; Ff, fin fold; FV, flexor ventralis; FVE, flexor ventralis externus; FVI, flexor ventralis inferior; HL, hypochordal longitudinalis; Hy, hypural; INT, interradialis; Nc, notochord; R, soft ray. Scale bar indicates 0.2 mm.

cles were observed. The flexor dorsalis, hypochordal longitudinalis, flexor ventralis and flexor ventralis externus were formed on the dorsal and ventral sides of the notochord. The flexor ventralis was located on the middle regions of the parhypural and hypural 1. The flexor dorsalis superior, flexor ventralis inferior and interradialis were recognized as small muscle fiber masses with myofibrils (Fig. 37).

In a 29.5 days old juvenile of 11.3 mm TL, the flexor ventralis elongated anteriorly and its ventral subdivision was slightly distinguished. The flexor dorsalis superior, flexor ventralis inferior and interradialis were considerably enlarged. The interradialis was divied into two muscles: the dorsal one was attached to the lateral face of the dorsal principal rays and the ventral one to the ventral principal rays. The caudal skeleton was not yet wholly covered by the caudal fin muscles (Fig. 38).

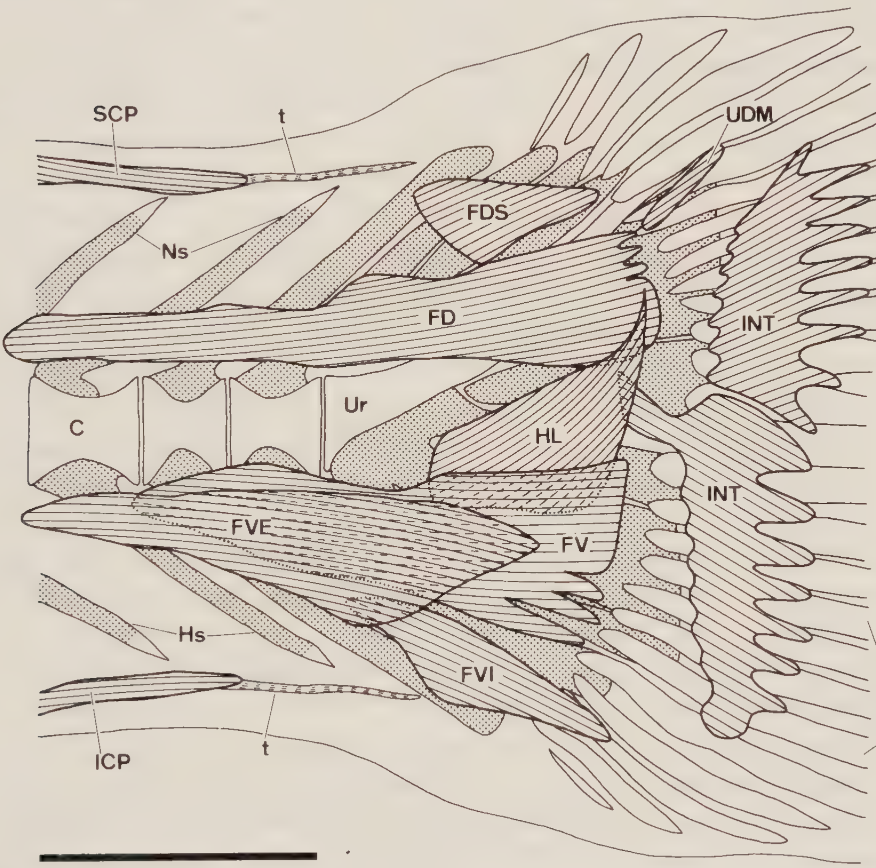


Fig. 38. Reconstruction of caudal fin muscles in *Pagrus major*, 11.3 mm TL. Lateral view. Cartilaginous and bony elements are partly shown. Hs, haemal spine; ICP, infracarinalis posterior; Ns, neural spine; SCP, supracarinalis posterior; t, tendon; UDM, undefined muscle; Ur, urostyle. For other abbreviations, see Fig. 37. Scale bar indicates 0.5 mm.

Discussion

The head muscles of *P. major* begin to differentiate shortly prior to onset of feeding. The adductor mandibulae, levator arcus palatini, adductor operculi, protractor hyoidei, sternohyoideus and other muscles recognized at this stage open and close the mouth and gill-cover and, therefore, are fundamentally indispensable for onset of feeding or respiration. The adductor mandibulae at this stage is mainly represented by a slender rod-like muscle, a compound of A1 and Aw. It is generally accepted that the adductor mandibulae acts for closing the mouth.[101] The anterior tip corresponding to Aw is attached to the Meckel's cartilage, so that the contraction of this muscle results in pulling up the lower jaw. A2 has been also formed as a small rod-like muscle, but it seems to be still functionless because of its free anterior and posterior tips. Opening of the mouth at this stage is mostly performed by a slender protractor hyoidei and sternohyoideus. The adductor operculi is a small and flat muscle. It acts in pulling the gill-cover medially for narrowing the branchial cavity.[101] The levator arcus palatini is a prominently large muscle, compared with other head muscles. FLÜCHTER[110] also indicated the existence of a large muscle probably corresponding to the levator arcus palatini in an early larva of herring. ISMAIL[104] reported that this muscle is thin and narrow in the buccal stage X of *Haplochromis elegans*, but grows enormously in length as well as in thickness as development proceeds. It plays a part in suspensorial abduction.[101] It is assumed that expanding the buccal and branchial cavities by the levator arcus palatini may supply the force for swallowing the water containing prey foods. Therefore, the function of this muscle as a swallowing pump may serve as an important feeding mechanism at early stages to supplement a thin adductor mandibulae.

The adductor arcus palatini at around onset of feeding is thin, situated posterior to the orbit, and elongates anteriorly as *P. major* larvae grow. Contrary to this result, ISMAIL[104] reported that this muscle appears in the anterior region and elongates posteriorly in *H. elegans*. It is indicative of developmental differences in the head muscles between different species.

For several days after onset of feeding, the composition of the head muscles and their sizes are almost the same. A few changes in the head muscle system are discernible in a 4.40 mm TL specimen, such as the differentiation of the levator operculi and tendinous attachment of the adductor mandibulae A2 (probably including A3 fibers) to the Meckel's cartilage. A2 is now able to function in closing the mouth. Conspicuous changes are recognizable in the head muscle system, especially in the adductor mandibulae, of 5.20 and 6.80 mm TL specimens. A1 and Aw are completely separated by 6.80 mm TL. The tendon of A1 is partly formed in a 5.20

mm TL specimen and completely connects A1 with the maxillary in a 6.80 mm TL specimen. ALEXANDER[111] suggested that A1 acts in the protrusion of the upper jaw during closing of the mouth. A1 is now able to perform this function. The hypertrophying of the adductor mandibulae increases the force for feeding by pulling up the lower jaw. Noticeable thickening of the protractor hyoidei and sternohyoideus beyond 5.20 mm TL is effective for allowing a wide opening of the mouth. The hyohyoidei inferioris, hyohyoidei abductores and hyohyoidei adductores are observed beyond 5.20 mm TL. The former two muscles act in fanning out the branchiostegal rays and the latter acts in folding upwards the branchiostegal membrane.[101] These functions are effective for expanding or narrowing the branchial cavity.

The differentiation of the head muscles is almost completed by 11.3 mm TL. But the relative sizes of respective muscles are somewhat different from those of a 110.5 mm TL specimen. The adductor mandibulae A2 is successively enlarged, while the levator arcus palatini becomes relatively smaller beyond this stage. It is suggested that the biting force by A2 particularly increases beyond this stage.

The pectoral fin muscles at around onset of feeding are simply composed of two flat muscles covering the fin plate cartilage. Larvae at this stage simultaneously move the tail and pectoral fins in swimming. It is undoubted that the pectoral fin muscles play an important role in propulsion by fanning of the fin plate and fin fold. The caudal fin muscles are entirely undifferentiated at this stage. The force of active tail beats is produced only by the undulation of the caudal region of the myotomal muscle.

The pectoral fin muscles have begun to differentiate from two flat muscles to its respective components at 6.80 mm TL. This differentiation seems to almost simultaneously occur with the separation of the fin plate cartilage to four actinosts and the ray formation, and by 11.3 mm TL, it has been already completed. The development of these structures may serve more complicated movement of the pectoral fin, e. g., for braking and controlling the vertical course of swimming, as mentioned in Chapter I.

The caudal fin muscle has began to differentiate between 4.60 and 6.00 mm TL. The formation seems to be parallel with the differentiation of the epural, parhypural, hypural and fin rays. The caudal fin muscle and caudal skeleton increase the force of tail beats for rowing water and also serve complicated movement of the caudal fin.

The present data is mostly limited to the morphological development of the head and fin muscles. Fiber types and their differentiation of these muscles could not be examined in detail. A few enzyme histochemical observations, however, were made in the course of the examination of the myotomal muscle and it was indicated that the pectoral, dorsal, anal and caudal fin muscles at early stages contain fibers showing intense staining for SDH and NADHD, like the red or pink fibers. Fiber

types of the head muscles are investigated in some adult fishes (e. g. ONO and KAUFMAN[112]), but there is very few literature on their differentiation during ontogeny.[107] To clarify the development of feeding and swimming mechanism, histochemical and electron microscopic studies on the differentiation of fiber types of the head and fin muscles would be required.

III. COMPARISON BETWEEN WILD AND LABORATORY-REARED SPECIMENS

BLAXTER[113] emphasized, in his review, the importance of morphological, behavioral, physiological and biochemical comparisons between wild and reared fish of the same species. ANRAKU and AZETA[23] compared the chemical components of wild and reared *P. major* juveniles and detected some differences between these two stocks. Figure 39 shows the cleared and stained juveniles of wild and reared *P. major* having almost the same size. These two specimens are apparently different even in external proportion, and suggest that the detailed morphological comparisons including internal organs between wild and reared stocks are needed. As mentioned before, these comparisons will be useful for the production of healthy and vigorous larvae.

This chapter is divided into two sections. The first is concerned with size com-



Fig. 39. Photograph of cleared and stained specimens of wild and reared *Pagrus major*. Top: wild specimen, 20.0 mm TL. Bottom: reared specimen, 20.0 mm TL. Scale bar indicates 5 mm.

parison in early morphological development between wild and reared *P. major*. The second is concerned with the detailed osteological comparison of meristic variation, bone abnormality and arrangement of vertebra-pteygiophore between wild and reared juveniles of this species.

1. Relationship between Size and Developmental Stages

Recently, some morphological and morphometric comparisons between wild and reared fish were made in teleosts. For example, ARTHUR[114] reported the differences in heart size between wild and reared larvae of *Engraulis mordax*. KITAJIMA[13] and MATSUMIYA *et al.*[24,25] recognized some external morphometric differences between late juveniles of wild and reared *P. major*.

In regard to the relationship between size and development, SHELBOURNE[115] suggested that reared larvae of plaice (*Pleuronectes platessa*) metamorphose at smaller sizes than wild ones. On the other hand, FUKUHARA and KUNIYUKI[18] compared the development of some characters between wild and reared *P. major* and recognized no distinct size difference in development except the formation of stripes. This section was devoted to reexamine the relationship between size and development of both wild and reared *P. major*.

Materials and methods

Specimens examined were two wild groups and two reared groups. Wild group 1: 86 specimens (range 5.00–14.15 mm TL) collected in Shijiki Bay (Nagasaki Prefecture) by the staffs of Seikai Regional Fisheries Research Laboratory in 1982. Wild group 2: 33 specimens (range 6.75–14.9 mm TL) collected in Yuya Bay (Yamaguchi Prefecture) by the staffs of Shimonoseki Branch of Seikai Regional Fisheries Research Laboratory in 1977. Reared group 1: 62 specimens (range 5.00–14.8 mm TL) reared in the Fisheries Research Station, Kyoto University in 1982. Reared group 2: 59 specimens (range 4.35–14.8 mm TL) reared in Fukui Prefectural Fisheries Farming Center in 1980.

Specimens fixed in formalin solution were cleared and stained, using the method of DINGERKUS and UHLER[26] for bone and cartilage.

Total length was measured after clearing and staining.

Observations were made under a stereomicroscope. The centrum and fin rays were used as the characters representing the developmental levels. The number of the centra was counted on only the cylindrical centrum forming "bony ring", without initially ossified spots of the centrum on both sides of the notochord. The anteriormost (first) centrum was not included in number, because it was difficult

to confirm its cylindrical condition as a bony ring in early stages. Counts of the dorsal, anal, pectoral, pelvic and caudal fin rays were made on the total number, the number of the spines (with sharp-pointed tip) and the number of the segmented soft rays. In the case of the caudal fin, ray counts were restricted to the principal rays. The posteriormost ray in the dorsal and anal fins (usually counted as one ray) consists of two rays articulated with one distal radial. In this section, each ray was treated as an independent one. The number of paired fin rays (pectoral and pelvic) was counted on left body side.

Results

Centrum

Figure 40 (A-D) shows the increment of the centra against total length in re-

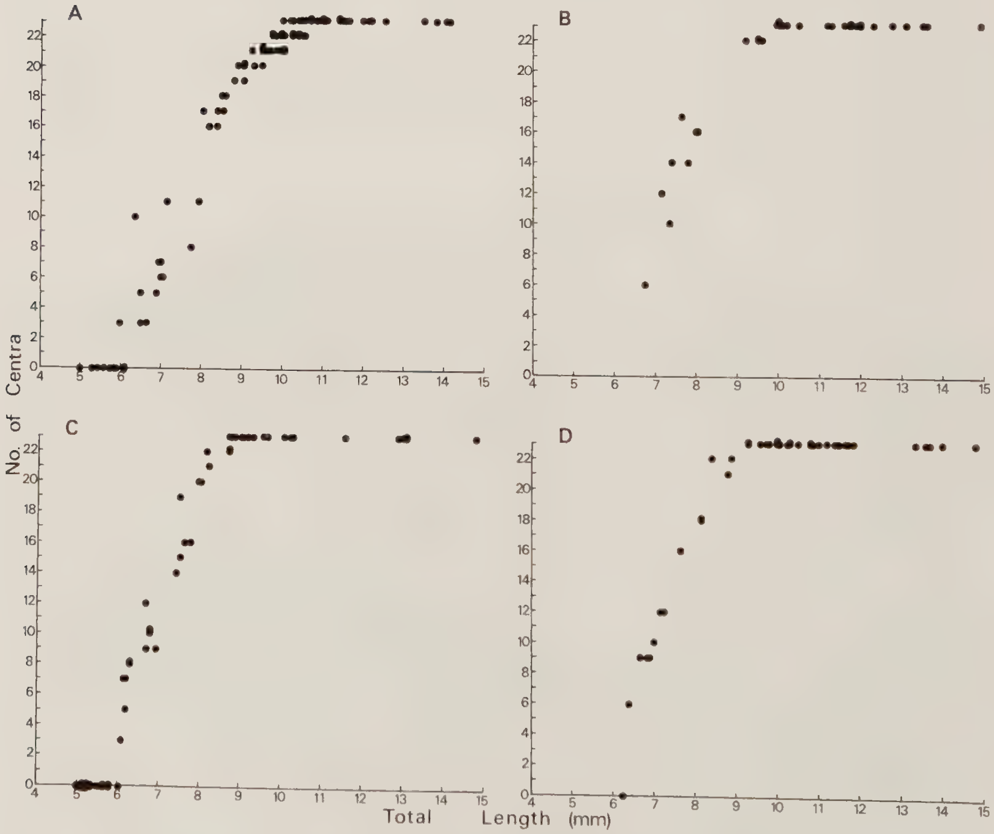


Fig. 40. Relationships between number of centra and total length in wild and reared *Pagrus major*. A: Wild group 1. B: Wild group 2. C: Reared group 1. D: Reared group 2. First centrum is not included in number.

spective four groups. The smallest specimen with the cylindrical centra was 6.00 (Wild 1), 6.75 (Wild 2), 6.10 (Reared 1) and 6.40 mm TL (Reared 2). A 6.75 mm TL specimen in Wild 2 was the smallest one examined in this group and had six centra. In Wild 1, the specimens smaller than about 8 mm TL showed relatively scattered plots of the number of the centra, contrary to the specimens beyond about 8 mm TL showing close plots. The full complement was first accomplished in a 10.0 (Wild 1), 9.95 (Wild 2), 8.75 (Reared 1) and 9.30 mm TL specimen (Reared 2).

Dorsal fin ray

Figure 41 (A-D) shows the increment of the dorsal fin rays. The smallest specimen with the ray rudiments was 6.35 (Wild 1), 7.15 (Wild 2), 6.15 (Reared 1) and 6.65 mm TL (Reared 2). The full complement of the rays including the ray

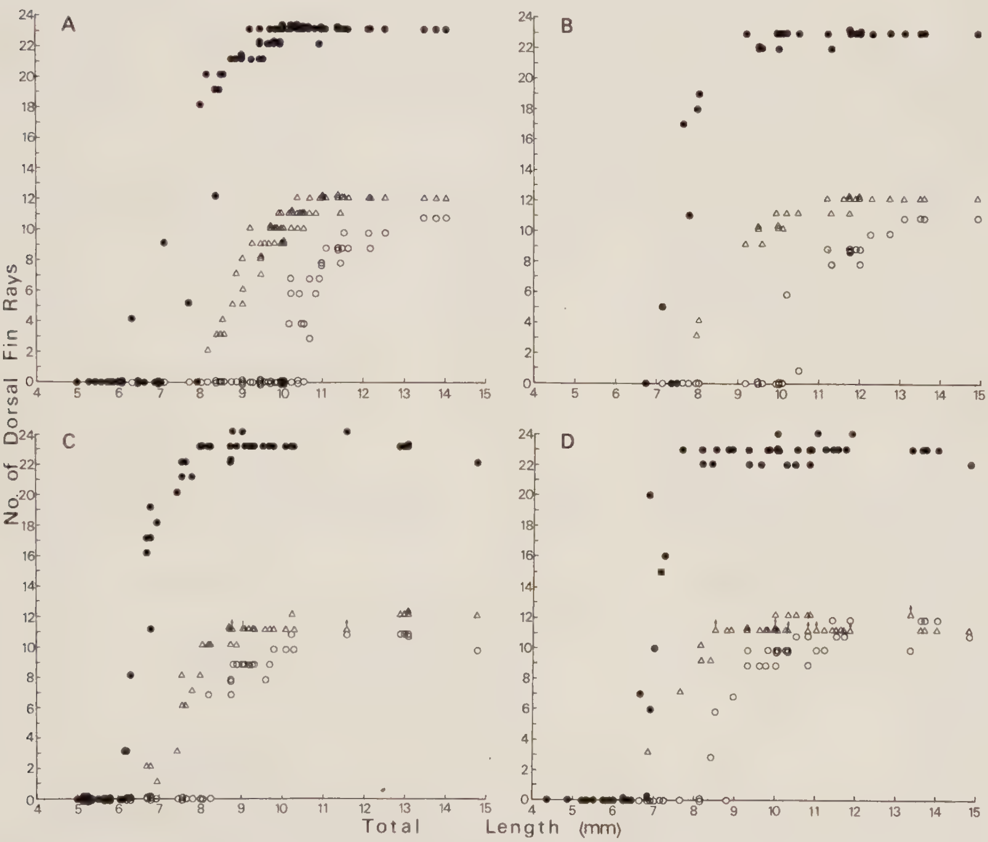


Fig. 41. Relationships between number of dorsal fin rays and total length in wild and reared *Pagrus major*. A: Wild group 1. B: Wild group 2. C: Reared group 1. D: Reared group 2. ●, total number of fin rays. △, number of spines. ○, number of segmented soft rays. ↑, small excess spine.

rudiments was first accomplished in a 9.25 (Wild 1), 9.20 (Wild 2), 8.00 (Reared 1) and 7.65 mm TL specimen (Reared 2). The rudiments of both spine and soft ray had the same structure, with actinotrichia at the distal tip, and later differentiated to the spine with sharp-pointed tip or the soft ray with segmentation.

The smallest specimen with the spines was 8.20 (Wild 1), 8.00 (Wild 2), 6.70 (Reared 1) and 6.85 mm TL (Reared 2). The full complement was first accomplished in a 10.4 (Wild 1), 11.2 (Wild 2), 10.25 (Reared 1) and 10.5 mm TL specimen (Reared 2). In reared groups, the number of the dorsal spines was considerably variable, contrary to wild groups. An excess small spine was often formed, just anteriorly to the original first one (small arrows in Fig. 41C, D). Some larger specimens had only 11 spines. The smallest specimen with 11 spines was 9.95 (Wild 1), 9.95 (Wild 2), 8.75 (Reared 1) and 8.50 mm TL (Reared 2).

The smallest specimen with the segmented soft rays was 10.2 (Wild 1), 10.2 (Wild 2), 8.20 (Reared 1) and 8.40 mm TL (Reared 2). The full complement was first accomplished in a 13.5 (Wild 1), 13.1 (Wild 2), 10.25 (Reared 1) and 10.5 mm TL specimen (Reared 2). The number of the segmented soft rays in larger specimens of reared groups was also variable.

Anal fin ray

Figure 42 (A-D) shows the increment of the anal fin rays. The smallest spec-

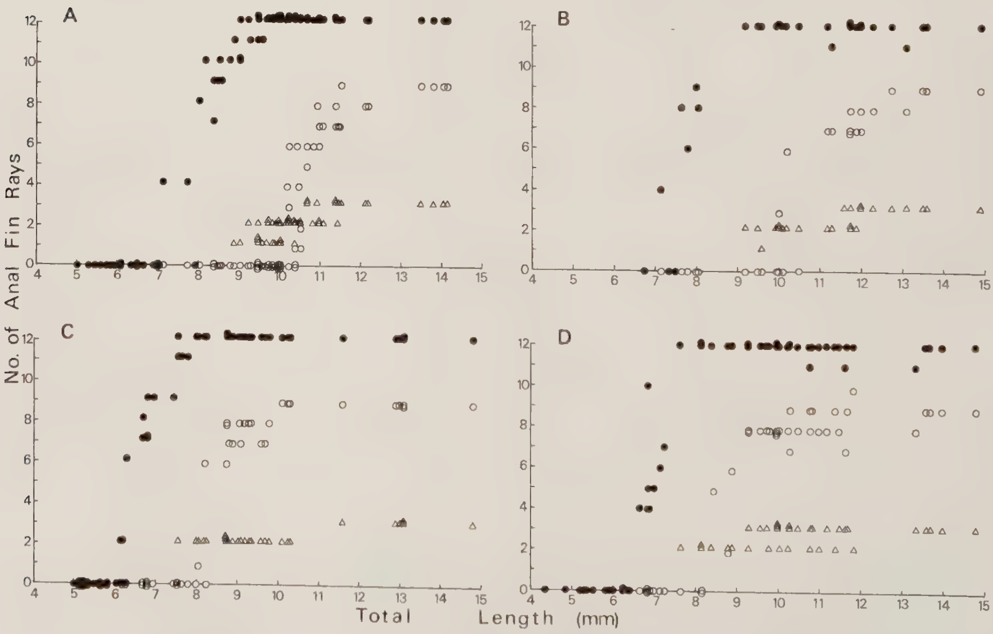


Fig. 42. Relationships between number of anal fin rays and total length in wild and reared *Pagrus major*. A: Wild group 1. B: Wild group 2. C: Reared group 1. D: Reared group 2. Symbols are the same in Fig. 41.

imen with the ray rudiments was 7.15 (Wild 1), 7.15 (Wild 2), 6.15 (Reared 1) and 6.65 mm TL (Reared 2). The full complement of the rays including the ray rudiments was first accomplished in a 9.05 (Wild 1), 9.20 (Wild 2), 7.55 (Reared 1) and 7.65 mm TL specimen (Reared 2).

The smallest specimen with the spines was 8.90 (Wild 1), 9.20 (Wild 2), 7.55 (Reared 1) and 7.65 mm TL (Reared 2). The full complement was first accomplished in a 10.7 (Wild 1), 11.6 (Wild 2), 11.6 (Reared 1) and 9.30 mm TL specimen (Reared 2).

The smallest specimen with the segmented soft ray was 10.2 (Wild 1), 10.0 (Wild 2), 8.05 (Reared 1) and 8.40 mm TL (Reared 2). The full complement was first accomplished in a 11.55 (Wild 1), 12.75 (Wild 2), 10.1 (Reared 1) and 10.3 mm TL specimen (Reared 2).

Pectoral fin ray

Figure 43 (A–D) shows the increment of the pectoral fin rays. The smallest specimen with the ray rudiments was 5.85 (Wild 1), 6.75 (Wild 2), 6.30 (Reared 1) and 6.25 mm TL (Reared 2). A 6.75 mm TL specimen in Wild 2, the smallest one examined in this group, had four ray rudiments. In Wild 1, the specimens smaller than about 8 mm TL showed relatively scattered plots of the number of the pectoral fin ray rudiments, compared with the specimens beyond about 8 mm TL.

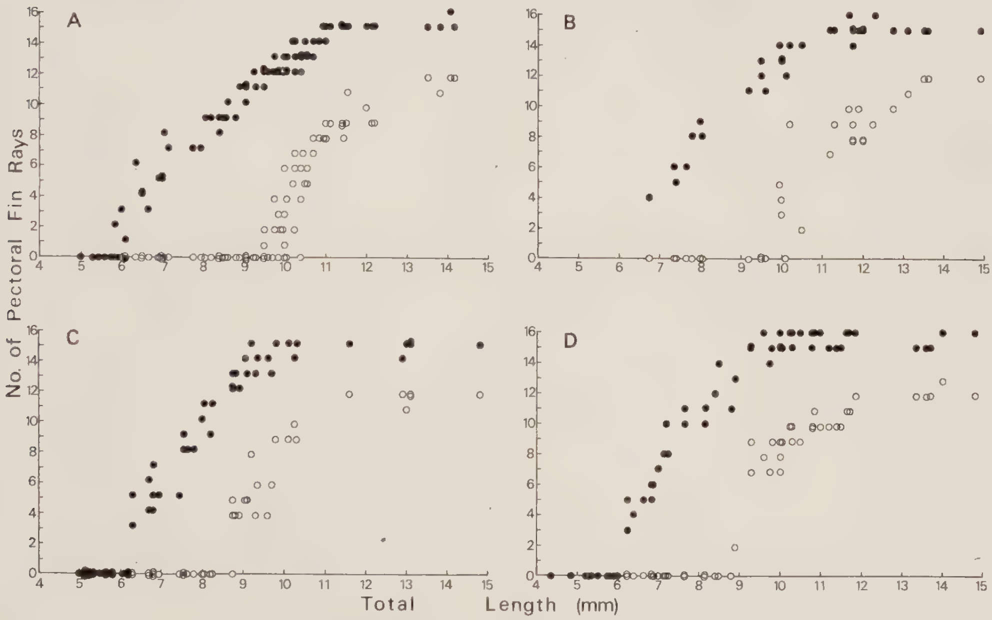


Fig. 43. Relationships between number of pectoral fin rays and total length in wild and reared *Pagrus major*. A: Wild group 1. B: Wild group 2. C: Reared group 1. D: Reared group 2. Symbols are the same in Fig. 41.

The full complement of the rays including the ray rudiments was first accomplished in a 10.95 (Wild 1), 11.2 (Wild 2), 9.20 (Reared 1) and 9.30 mm TL specimen (Reared 2). In Reared 2, half of the specimens beyond 9 mm TL had 16 rays (usually 15).

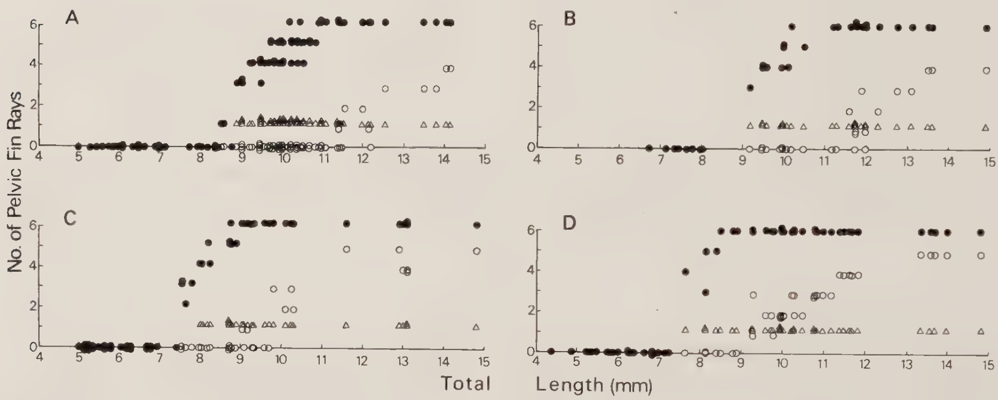


Fig. 44. Relationships between number of pelvic fin rays and total length in wild and reared *Pagrus major*. A: Wild group 1. B: Wild group 2. C: Reared group 1. D: Reared group 2. Symbols are the same in Fig. 41.

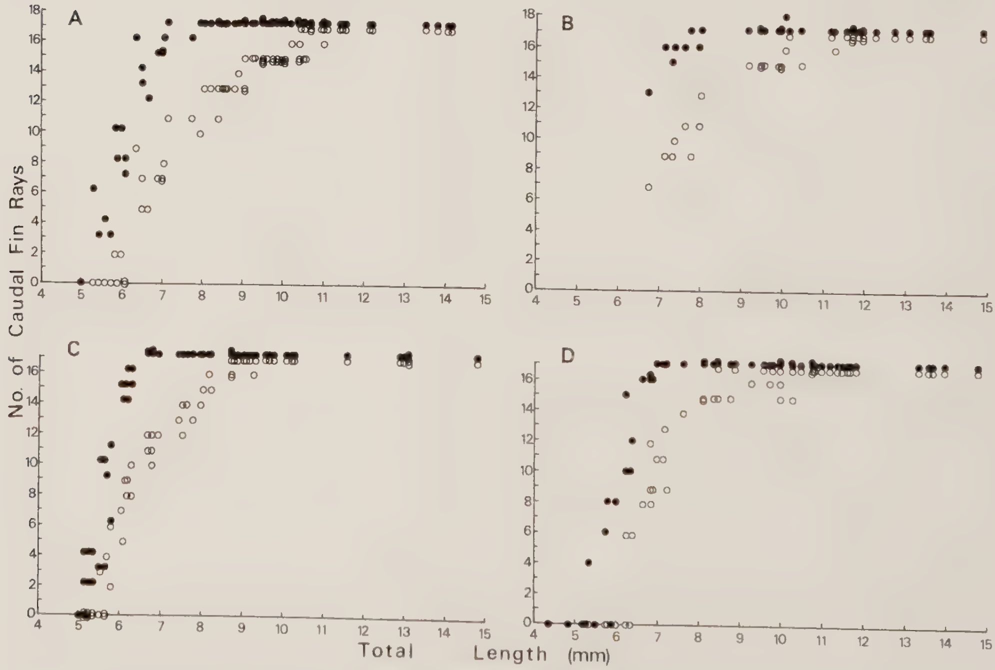


Fig. 45. Relationships between number of caudal fin rays and total length in wild and reared *Pagrus major*. A: Wild group 1. B: Wild group 2. C: Reared group 1. D: Reared group 2. Symbols are the same in Fig. 41.

The smallest specimen with the segmented soft rays was 9.50 (Wild 1), 9.95 (Wild 2), 8.75 (Reared 1) and 8.90 mm TL (Reared 2). The full complement was not accomplished even in the largest specimen of respective four groups. The smallest specimen with 12 segmented soft rays was 13.5 (Wild 1), 13.5 (Wild 2), 11.6 (Reared 1) and 11.85 mm TL (Reared 2).

Pelvic fin ray

Figure 44 (A-D) shows the increment of the pelvic fin rays. The smallest specimen with the ray rudiments was 8.55 (Wild 1), 9.20 (Wild 2), 7.55 (Reared 1) and 7.65 mm TL (Reared 2). The full complement of the rays including the ray rudiments was first accomplished in a 10.2 (Wild 1), 10.2 (Wild 2), 8.75 (Reared 1) and 8.50 mm TL specimen (Reared 2).

The smallest specimen with the spines was 8.90 (Wild 1), 9.20 (Wild 2), 8.00 (Reared 1) and 7.65 mm TL (Reared 2).

The smallest specimen with the segmented soft rays was 11.4 (Wild 1), 11.6 (Wild 2), 9.05 (Reared 1) and 9.30 mm TL (Reared 2). In reared groups, the full complement was first accomplished in a 11.6 (Reared 1) and 13.35 mm TL specimen (Reared 2), although not accomplished even in the largest specimen of respective

Table 2. Fish sizes (total length in mm) reaching first appearance (left) and full complement (right) of centrum and fin ray in wild and reared groups of *Pagrus major*.

	Wild 1	Wild 2	Reared 1	Reared 2
Centrum	6.00 - 10.0	6.75 - 9.95	6.10 - 8.75	6.40 - 9.30
Dorsal fin				
ray rudiment	6.35 - 9.25	7.15 - 9.20	6.15 - 8.00	6.65 - 7.65
spine	8.20 - 10.4	8.00 - 11.2	6.70 - 10.25	6.85 - 10.5
segmented ray	10.2 - 13.5	10.2 - 13.1	8.20 - 10.25	8.40 - 10.5
Anal fin				
ray rudiment	7.15 - 9.05	7.15 - 9.20	6.15 - 7.55	6.65 - 7.65
spine	8.90 - 10.7	9.20 - 11.6	7.55 - 11.6	7.65 - 9.30
segmented ray	10.2 - 11.55	10.0 - 12.75	8.05 - 10.1	8.40 - 10.3
Pectoral fin				
ray rudiment	5.85 - 10.95	6.75 - 11.2	6.30 - 9.20	6.25 - 9.30
segmented ray	9.50 - 13.5*	9.95 - 13.5*	8.75 - 11.6*	8.90 - 11.85*
Pelvic fin				
ray rudiment	8.55 - 10.2	9.20 - 10.2	7.55 - 8.75	7.65 - 8.50
spine	8.90	9.20	8.00	7.65
segmented ray	11.4 - +	11.6 - +	9.05 - 11.6	9.30 - 13.35
Caudal fin				
ray rudiment	5.30 - 7.15	6.75 - 7.80	5.15 - 6.70	5.35 - 7.00
segmented ray	5.85 - 10.45	6.75 - 10.2	5.55 - 8.75	6.25 - 8.50

*, fish sizes with 12 segmented rays; +, uncompleted in the largest specimen examined.

wild groups.

Caudal fin ray

Figure 45 (A-D) shows the increment of the principal caudal rays. The smallest specimen with the ray rudiments was 5.30 (Wild 1), 6.75 (Wild 2), 5.15 (Reared 1) and 5.35 mm TL (Reared 2). A 6.75 mm TL specimen in Wild 2, the smallest one examined in this group, had six ray rudiments and seven segmented rays. The full complement of the rays including the ray rudiments was first accomplished in a 7.15 (Wild 1), 7.80 (Wild 2), 6.70 (Reared 1) and 7.00 mm TL specimen (Reared 2).

The smallest specimen with the segmented rays was 5.85 (Wild 1), 6.75 (Wild 2), 5.55 (Reared 1) and 6.25 mm TL (Reared 2). The full complement was first accomplished in a 10.45 (Wild 1), 10.2 (Wild 2), 8.75 (Reared 1) and 8.50 mm TL specimen (Reared 2).

Table 2 shows fish sizes reaching the first appearance and full complement of the centra and fin rays in respective wild and reared groups.

Discussion

Figures 40-45 and Table 2 apparently indicate that the development of the centrum and fin rays in reared specimens of *P. major* proceeds at smaller sizes than in wild specimens. It is possible that lengths of small wild specimens are more or less shortened due to body shrinkage during collection by larval nets.[116] Taking this body shrinkage into consideration, actual size differences in development between wild and reared specimens migtht be larger than those shown here.

FUKUHARA and KUNIYUKI[18] compared the development of the fin rays, scale and black stripe on the body sides between wild and reared *P. major*, and recognized no distinct size difference except the formation of black stripes. In the development of the fin rays, they observed only segmentation and branching of the soft rays at relatively larger sizes. Therefore, it is suggested that such a results may be derived by insufficient number of small wild specimens examined to detect slight size differences between wild and reared specimens at smaller size.

In regard to the relationship between size and development, OKIYAMA[117] suggested that the size at which whole body sides of *Paralichthys olivaceus* are covered by scales is smaller in reared specimens than in wild specimens. SHELBOURNE[115] mentioned that reared larvae of plaice (*Pleuronectes plattessa*) metamorphose at smaller sizes than wild larvae. MINAMI[118] pointed out that reared *P. olivaceus* larvae settle to the bottom at smaller sizes than wild larvae. In wild *P. olivaceus* larvae, MINAMI *et al.*[119] reported that the size at metamorphosis tends to become smaller in later spawning season than in earlier season and suggested the influence of tem-

perature as a cause for this phenomenon. SEIKAI *et al.*[120] reared *P. olivaceus* larvae at three different temperatures, 13, 16 and 19℃, and indicated that larvae reared at 13℃ metamorphose at larger size than others. They suggested that, at low temperature, differentiation of organs or metamorphosis may be delayed than the elongation of body length. MATSUMIYA *et al.*[24] mentioned that the temperature at which larvae grow is different between natural and rearing conditions, i. e., larval production for marine fish farming in Japan is generally performed under higher temperature than the natural environment for the aim of accelerating growth. Therefore, it is likely that the differrence in temperature may be one of the causes which influence size differrences at the same developmental stages between wild and reared fishes in early ontogeny.

On the other hand, my obervations indicate that the completion of the osteological structures in *P. major*, characterized by the formation of Ac 7 and 8 of the caudal skeleton and the ossification of the middle radials of the dorsal and anal fin-supports, is accomplished at no longer than 45 mm TL in wild specimens, but at larger sizes, 56-72 mm TL or a little longer, in reared specimens (see Chapter I). This is in contrast to the early development of the centrum and fin rays. Usually, *P. major* larvae are reared in tanks and fed on living organisms, up to about 10-15 mm TL. Beyond this size, fishes are transfered to culture-nets set in the sea and fed on minced meat of fish, shrimp or mollusk. Such changes in rearing condition may influence the differentiation of organs and body growth of juveniles, and cause size differences at the stage of completion of the osteological structures between wild and reared *P. major*.

Size differences at the same developmental stages between wild and reared *P. major* presented here are only related to their osteological development. ARTHUR[114] indicated that reared larvae of *Engraulis mordax* have a larger heart than wild ones of the same sizes. Therefore, it would be necessary to also examine the relationship between size and developmental stages of both wild and reared fishes in other organs.

2. Meristic Variation and Bone Abnormality in Juveniles

There has been numerous literature concerning the meristic variation in teleosts, especially in the number of the vertebrae (see, FOWLER[121]), because meristic characters have significant use for identification of species and populations. But detailed comparisons of the meristic variations in many characters between wild and reared specimens of the same species were relatively few. In regard to the anomalies of fishes, over a thousand papers have been presented, mainly in adults (see, DAWSON[122-124]). Recently, information on the anomalies of reared fishes has been

increasing (e. g. KITAJIMA,[13] TAKASHIMA,[14,55] HOUDE,[125] TAKASHIMA *et al.*,[126] BARAHONA-FERNANDES,[127] SHIMIZU and FUJITA,[128] etc). The majority of such information is related to serious anomalies which can be observed even from external morphology. Detailed examinations including slight anomalies of the internal characters, however, have been little, particularly from the viewpoint of comparison between wild and reared fishes of the same species.

This section was devoted to clarify the meristic variation, bone abnormality and arrangement of vertebra-pterygiophore in both wild and reared juveniles of *P. major*.

Materials and methods

Specimens examined were two wild groups and four reared groups. Wild group A: 100 specimens (range 21.0-51.5 mm TL) collected in Shijiki Bay (Nagasaki Prefecture) by the staffs of Seikai Regional Fisheries Research Laboratory in 1977. Wild group B: 59 specimens (range 12.3-28.6 mm TL) collected in the coastal area of Kumihama (Kyoto Prefecture) by the staffs of Kyoto Institute of Oceanic and Fishery Science in 1980 and 1981. Reared group A1: 50 specimens (range 14.0-20.9 mm TL) reared in the Fisheries Research Station, Kyoto University in 1981. Reared group A2: 50 specimens (range 8.15-12.5 mm TL) reared from eggs spawned by the same parental stock as Reared A1, but reared in another tank. Reared group B: 50 specimens (range 12.2-20.1 mm TL) reared in Fukui Prefectural Fisheries Farming Center in 1981. Reared group C: 25 specimens (range 31.0-72.0 mm TL) reared in Aquaculture Research Laboratory, Nagasaki Prefectural Institute of Fisheries in 1977.

Specimens fixed in 10% formalin solution were cleared and stained by the bone staining methods of HOLLISTER[129] for Wild A and TAYLOR[31] for Reared C, and by the bone and cartilage staining method of DINGERKUS and UHLER[26] for other groups.

Total length was measured after clearing and staining.

Observations were made under a stereomicroscope. Meristic characters such as the numbers of the vertebrae, pleural and dorsal ribs and fin rays were counted. The number of the vertebrae was counted in all groups. In reared groups, accurate counting of the vertebral number was difficult because a large number of specimens had fused centra. GABRIEL[130] suggested that the counting of the arch elements is more reliable than counting the centra. So, a centrum with two neural spines and/or two haemal spines was considered to be formed by fusion and counted as two, even if the centrum itself had no indication of fusion in appearance. The numbers of the pleural and dorsal ribs were counted on relatively larger specimens of Wild A and Reared C, because of their late attainment of full complements. The num-

bers of the fin rays were counted on two wild groups and three reared groups, except Reared A2 in which the fin rays were still in the course of formation. Two rays articulated with the posteriormost distal radial in the dorsal and anal fins were counted as one in this section. The numbers of the pectoral and pelvic fin rays were counted on the left body side. The arrangement of vertebra-pterygiophore was examined in Wild A, Wild B, Reared A1 and Reared B. Bone abnormality was examined in all groups.

Results

Meristic variation

The number of the vertebrae in all spariform fishes is 10+14=24.[131] In Wild A, 98 specimens (98%) had 24(10+14) vertebrae. Two specimens (2%) were in 25(10+15), including one specimen (1%) with a fused caudal centrum as two. All specimens of Wild B had 24(10+14) vertebrae. In Reared A1, 48 specimens (96%) had 24(10+14) vertebrae, including 10 specimens (20%) with a fused centrum. Two specimens (4%) were in 23(10+13). Reared A2 consisted of relatively smaller specimens, in which a few preural centra of some specimens were still in the course of formation. Most specimens seemed to have 24(10+14) vertebrae, judged from arch elements, but 36 specimens (72%) had one or more fused centra. All specimens of Reared B had 24(10+14) vertebrae, including one specimen (2%) with a fused centrum. In Reared C, 23 specimens (92%) had 24(10+14) vertebrae, including four specimens (16%) with a fused centrum. Two specimens (8%) were in 24(9+15).

All 17 specimens examined (range 27.0-51.5 mm TL) of Wild A had eight pairs of the pleural ribs. In 25 specimens (range 31.0-72.0 mm TL) of Reared C, 21 specimens had eight pairs of the pleural ribs. Three specimens had seven pairs and one specimens was with seven and eight on either body side. The number of the dorsal ribs was more variable in wild specimens than other meristic characters. 13 specimens of Wild A had 12 pairs of the dorsal ribs. Three specimens were with 11 and 12, and one specimen with 12 and 13 on either body side. On the other hand, only two specimens had 12 pairs of the dorsal ribs in Reared C. Others had 11 pairs (11 specimens), 11 and 12 (11 specimens), and 12 and 13 (one specimen) on either body side.

Figure 46 shows variations of the number of the dorsal fin rays. In two wild groups, the majority of specimens (96% in Wild A and 94.9% in Wild B) had the fin ray formula of XII-10, consisting of 12 spines and 10 soft rays. A few specimens with XI-10, XII-9, XII-11 or XIII-9 rays were observed. In contrast, the number of the dorsal fin rays in reared groups was considerably variable and speci-

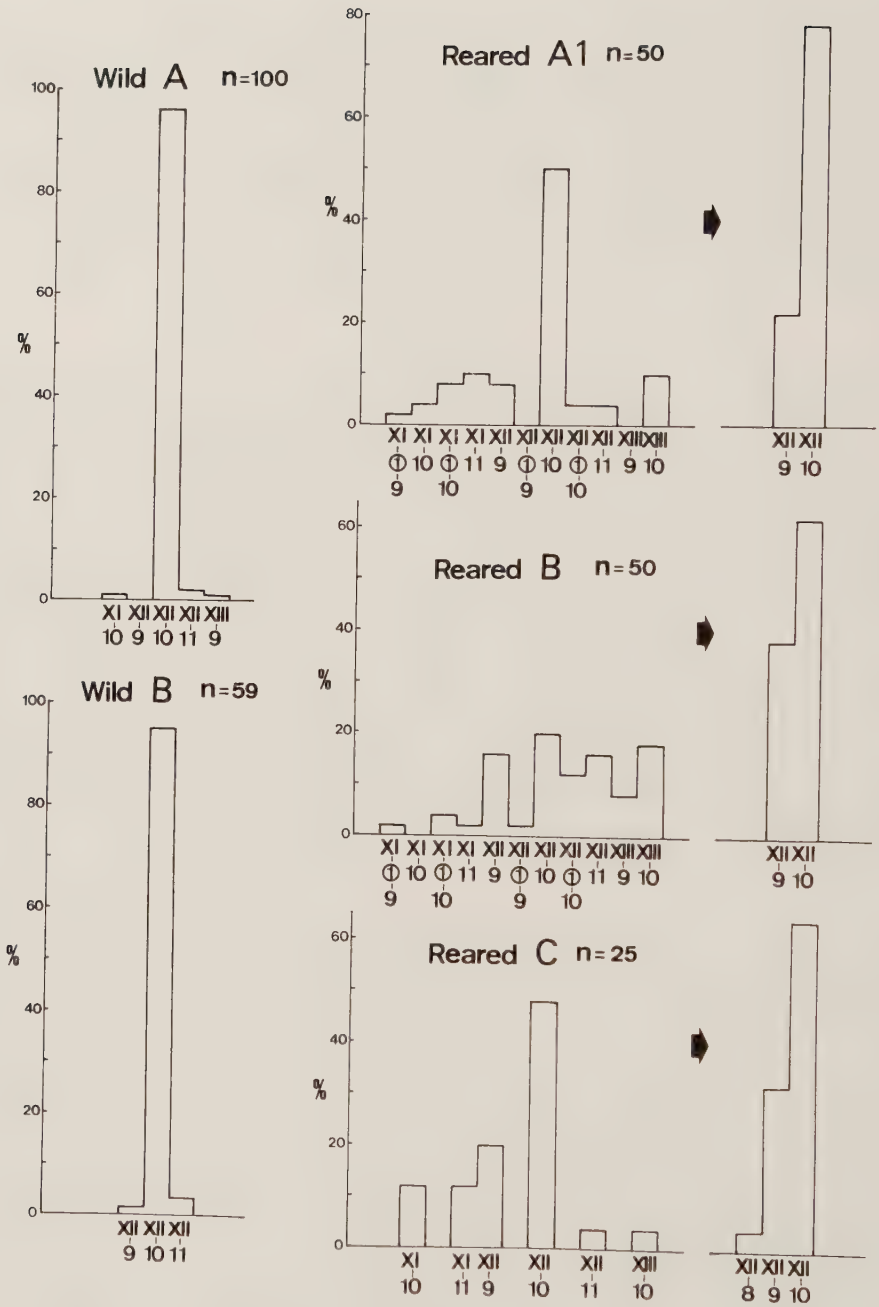


Fig. 46. Frequency distribution (%) of number of dorsal fin rays in respective wild and reared groups. Number in circle indicates undifferentiated ray. Arrows show recounted frequency distribution to eliminate influence of abnormal rays in reared groups.

mens with XII-10 rays were not more than 50% (the minimum was 20% in Reared B). These variations in appearance included following two abnormal developments in the dorsal fin rays. One was due to an excess small spine which was often formed, just anteriorly to the original first one (arrow in Fig. 48G). The other was because the original posteriormost spine judged from the arrangement of vertebra-pterygiophore often exhibited intermediate structure between the spine and

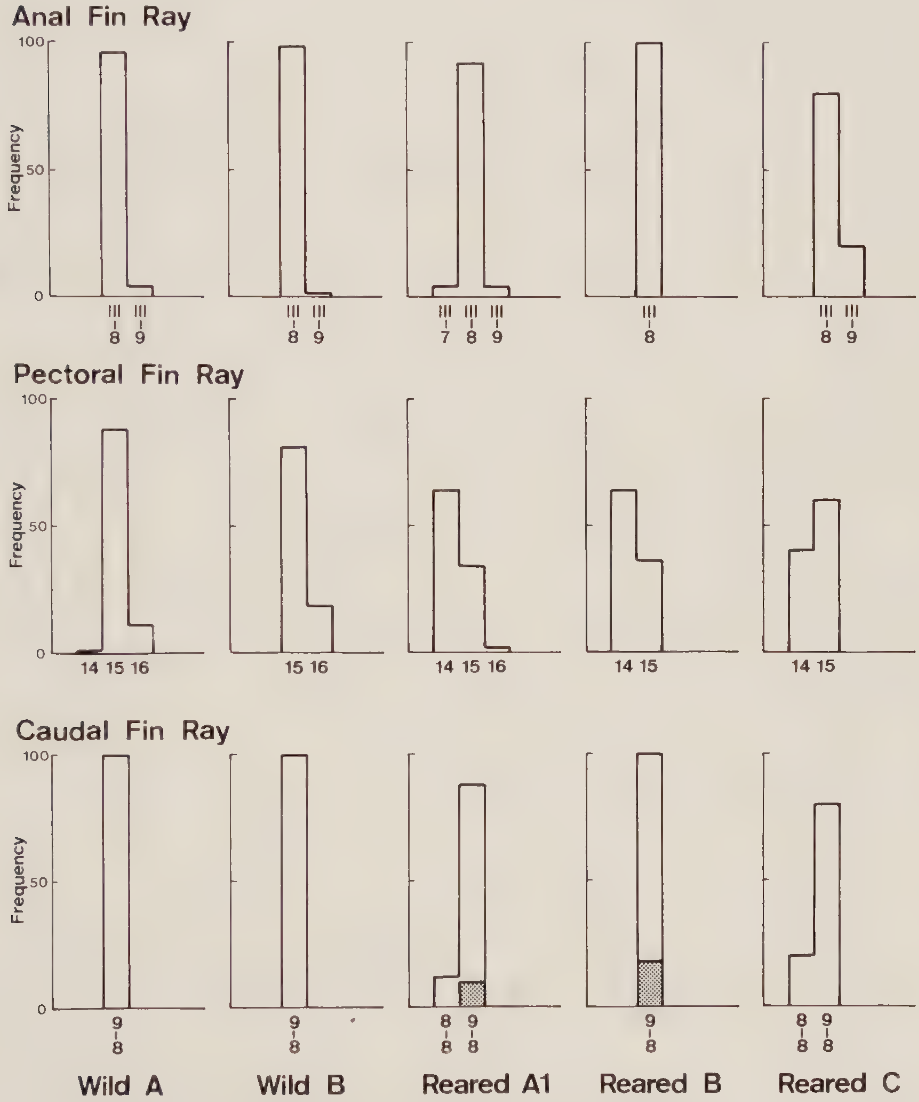


Fig. 47. Frequency distribution (%) of number of anal, pectoral and caudal fin rays in respective wild and reared groups. Stippled bar indicates frequency of fusion between rays in caudal fin.

soft ray, i.e., a thin and long spine or a soft ray with a few segmentations only on the distal tip (Fig. 48A). In some specimens, the ray was still undifferentiated to the spine with sharp-pointed tip or the soft ray with segmentation even at the size where differentiation should be usually completed. Such undifferentiated rays are shown with circle in Reared A1 and Reared B (Fig. 46), but not observed in Reared C which consisted of larger specimens, where they probably grew to be intermediate rays. Recounting of the dorsal fin rays was made to eliminate the influence of such abnormal developments from natural meristic variations: the excess small spine anterior to the original first one was not included and the original posteriormost spine was counted as a spine even if it was intermediate or undifferentiated. The results are shown by arrows in Fig. 46. Specimens with XII-9 rays, indicating decrease of the soft rays, reached from about 20% to 40% in reared groups. Figure 47 shows variations in the numbers of the anal, pectoral and caudal fin rays. Variations in the number of the anal fin rays in reared groups were considerably fewer than those of the dorsal fin rays, except Reared C. Specimens with III-8 rays reached 96% in Wild A, 98.3% in Wild B, 92% in Reared A1 and 100% in Reared B, while Reared C included 20% specimens with III-9 rays. The number of the pectoral fin rays in wild groups was more variable than the numbers of other fin rays. 11.1% specimens in Wild A and 18.6% in Wild B had 16 rays, although a large number of specimens had 15 rays. In reared groups, however, specimens with 15 rays were only 34% in Reared A1, 36% in Reared B and 60% in Reared C: the rest was occupied by specimens with 14 rays. The number of the pelvic fin rays was not variable in both wild and reared groups, in which all specimens had I-5 rays, although KOHNO *et al.*[15] described only 5 rays even in the largest specimen examined. The number of the principal caudal rays was not variable in wild groups, in which all specimens had 17(9+8) rays. In reared groups, 12% specimens in Reared A1 and 20% in Reard C had 16(8+8) rays. 10% specimens in Reared A1 and 18% in Reared B possessed a fused ray counted as two which are shown by stippled bars in Fig. 47.

Bone abnormality

The main types of bone abnormality observed were as follows:

(1) Upper and lower jaws. Both the upper and lower jaws or only the lower jaw were shortened, accompanied with reduction or deformation of the dentary, premaxillary, maxillary and palatine (Fig. 48B, C). Shortening of only the upper jaw was very few.

(2) Centrum. The first centrum was reduced, showing a triangle shape from the lateral view, accompanied with deformation of several posterior centra (Fig. 48D). Successive two or three centra were deformed not only in the anterior region but

in other regions of the vertebrae. The fusion of the centra was mainly concentrated in the posterior caudal vertebrae. The fusion differed in degree: the fused face between two centra was recognizable in sowe cases (Fig. 48E), but a somewhat long centrum with two neural and/or two haemal spines was formed without a distinct fused face in other cases. A reduced triangle centrum with only haemal spine was often formed just anterior to the urostyle.

(3) Neural and haemal arches and spines. The tip of the neural and haemal spines was branched anteriorly and posteriorly. Adjoining neural or haemal spines were fused to each other (Fig. 48E, F). The neural and haemal spines were shortened (Fig. 48F), and occasionally both arch and spine were wholly lacking. The fusion, shortening and lack of the neural and haemal arches and spines were frequently accompanied with fusion or reduction of the centra.

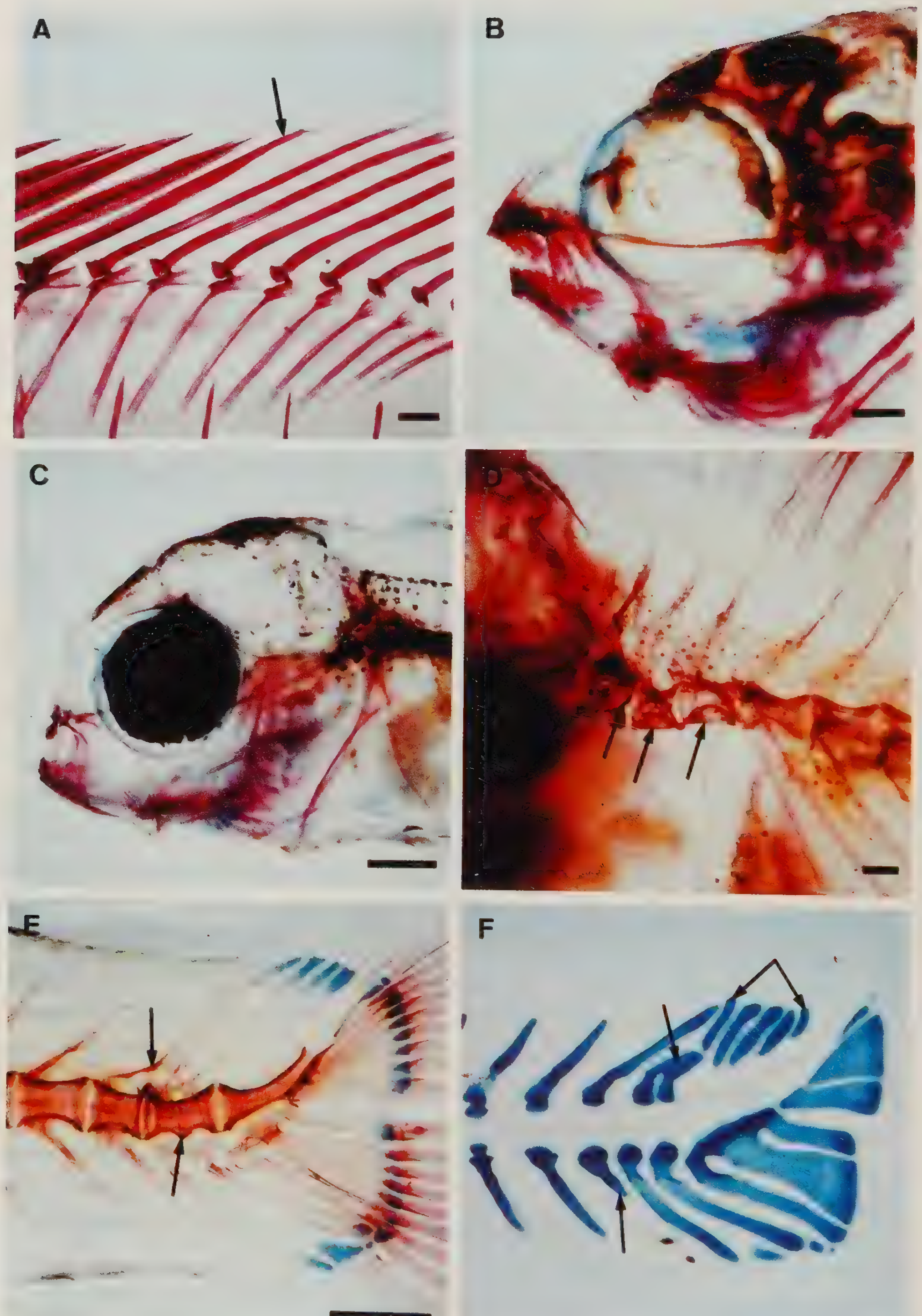
(4) Predorsal. The shortening, branching and fusion of the predorsals were observed. The increase or decrease in number of the predorsals were also seen. They do not seem to be natural meristic variations but extreme cases derived from branching or fusion.

(5) Dorsal and anal pterygiophores. The first proximal radial of the dorsal fin was branched at the tip (Fig. 48G) or sometimes wholly divided into two proximal radials showing normal conditions in appearance (Fig. 48H). In many cases, branching of the first proximal radial was accompanied with the formation of an excess small spine (arrow in Fig. 48G), just anterior to the original first one, so that the branched first proximal radial had three spines with secondary articulation and one spine with serial articulation. The deformed first proximal radial was distinguishable from the normal one even in cartilaginous state because a relatively large process or separated cartilage from the first proximal radial was formed (Fig. 48I). The first proximal radial of the anal fin was not deformed like that of the dorsal fin. The deformation and fusion of the dorsal and anal pterygiophores were occasionally observed, especially in a reared group in which all specimens had many deformed proximal radials in the trunk region (Fig. 48J).

(6) Parhypural and hypural. The parhypural and hypural 1 and 2 were fused, mostly between the parhypural and hypural 1, and between the hypural 1 and 2. The bases of the parhypural and hypural 1 and 2 were normally fused by cartilage in early stages (Fig. 10A, B, C), but the above cases were by fusion of ossified structures. The hypural 5 was frequently lacking.

(7) Epural. The shortening, branching and fusion of the epurals were observed. The increase or decrease in the number of epurals in appearance did not seem to be natural meristic variations, but were probably derived from branching or fusion, like the predorsals.

(8) Branchiostegal ray. The first and second branchiostegal rays were fused at


A
B
C
D
E
F

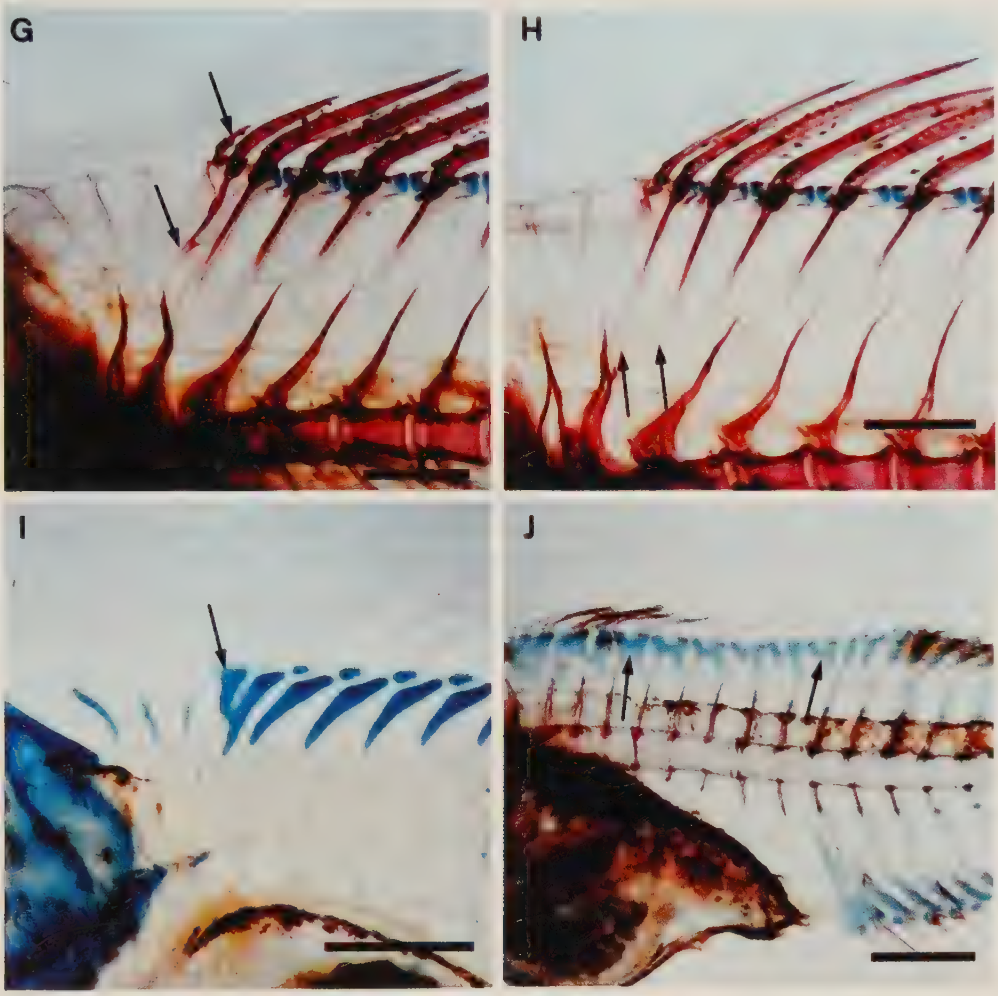


Fig. 48. Photographs showing abnormal bones in cleared and stained specimens of reared *Pagrus major*. A: 47.0 mm TL with intermediate ray having a few segmentations on only distal tip (arrow). B: 16.6 mm TL with shortened lower jaw. C: 9.70 mm TL with both shortened upper and lower jaws. D: 21.1 mm TL with deformed anterior three centra (arrows). E: 17.1 mm TL with fused caudal centra and fused neural arches (arrows), etc. F: 8.75 mm TL with fused neural arches, shortened haemal spine and over-formed epurals (arrows). G: 16.0 mm TL with branched first proximal radial and an excess small spine (arrows). H: 16.8 mm TL with completely separated first proximal radial (arrows). I: 8.10 mm TL with a large process from first proximal radial (arrow). J: 7.50 mm TL with deformed and fused proximal radials in dorsal fin-supports (arrows). Scale bars indicate 0.5 mm.

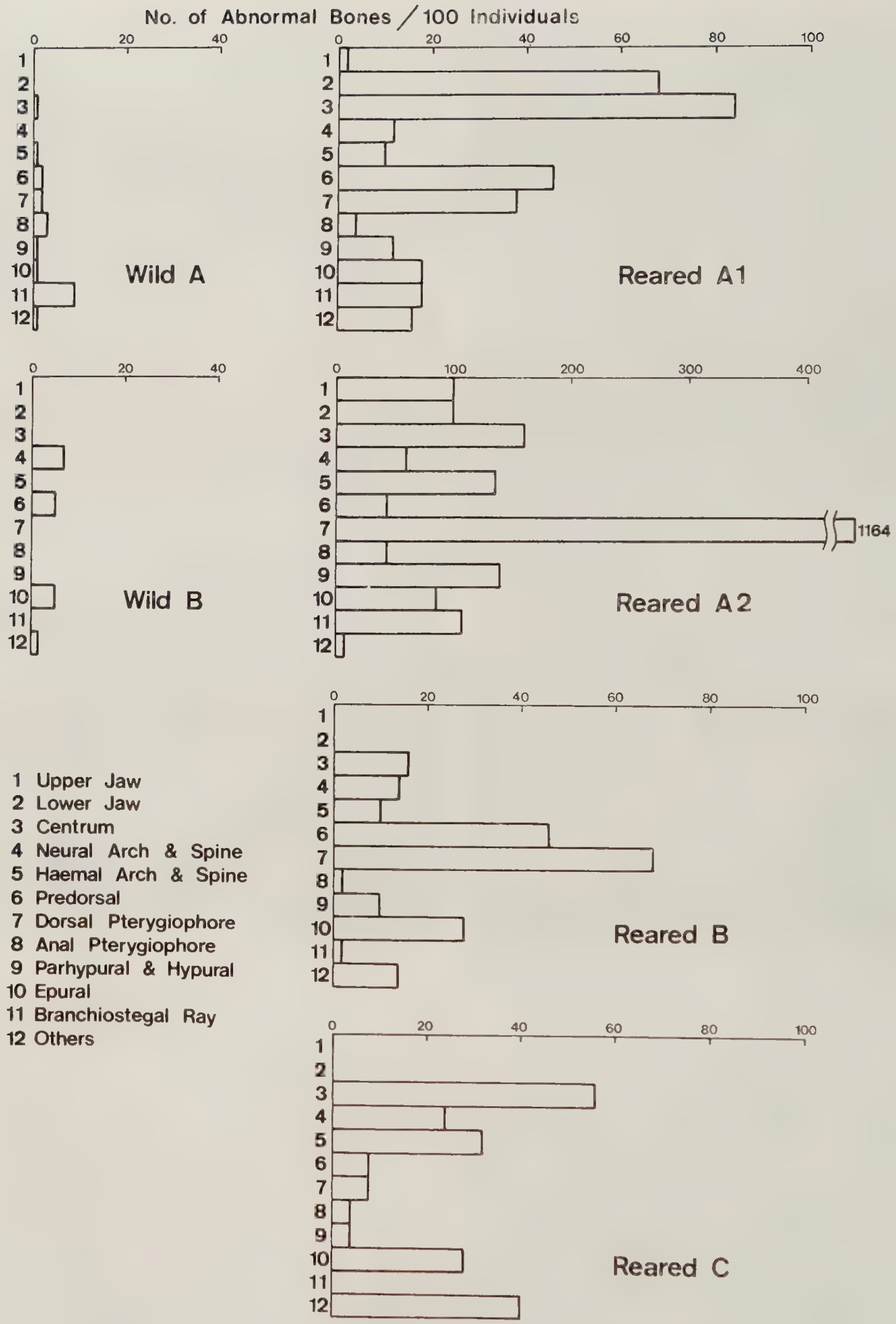


Fig. 49. Number of abnormal bones divided into 12 types per calculated 100 individuals in respective wild and reared groups.

the bases. A thick ray was often formed by complete fusion of those rays. The increase and decrease in the number of branchiostegal rays was also observed and included as bone abnormality.

(9) Others. There were a few examples showing the shortening, separation and lack of the pleural ribs. In rare cases, the tips of the pleural ribs except the eighth pair were ossified and sharp-pointed, while their tips were usually covered by cartilages. The ventralmost actinost of the pectoral fin was deformed, sometimes with a foramen in it. The accessory cartilage Ac 4 was fused with Ac 3 or Ac 5, and Ac 4 and Ac 5 were respectively separated into small cartilages.

Bone abnormality was rarely seen in the head skeleton, and the pectoral and pelvic fin-supports, except for the shortening of the upper and lower jaws and the deformation of the ventralmost actinost.

Figure 49 shows the number of abnormal bones divided into 12 types per calculated 100 individuals in respective wild and reared groups. In Wild A, there were nine cases of abnormal (including meristic variations in appearance) branchiostegal ray per 100 individuals in which six cases had seven rays. Other abnormal bones were very few. In Wild B, abnormal bones were discerned in the neural arch and spine (6.8 cases per calculated 100 individuals), predorsal (5.1 cases), epural (5.1 cases) and others (1.7 cases). The numbers of abnormal bones in all reared groups were considerably more than those in wild groups. In Reared A1, abnormal bones were particularly concentrated on the lower jaw (68 cases per calculated 100 individuals), centrum (84 cases), predorsal (46 cases) and dorsal pterygiophore (38 cases). Specimens of Reared A2, grown from eggs spawned by the same parental stock as Reared A1, had numerous abnormal bones of almost all types. Abnormality in the dorsal pterygiophore was considerably high, 1164 cases per calculated 100 individuals. All specimens examined had shortened upper and lower jaws and malformation was also concentrated on the centrum (160 cases), haemal arch and spine (136 cases), parhypural and hypural (130 cases), branchiostegal ray (108 cases) and so on. In Reared B, high abnormality occurred in the predorsal (46 cases per calculated 100 individuals), dorsal pterygiophore (68 cases) and epural (28 cases). In Reared C, abnormal bones were concentrated on the centrum (56 cases per calculated 100 individuals), neural arch and spine (24 cases), haemal arch and spine (32 cases), epural (28 cases), and others (40 cases, mostly in the pleural rib).

Figure 50 shows the frequency of specimens having at least one abnormal bone and the average number of abnormal bones per individual in respective wild and reared groups. The results in two wild groups were very similar, in which about 15% specimens had abnormal bones and the average number of abnormal bones was about 0.2 per individual. On the other hand, the percentage of specimens with

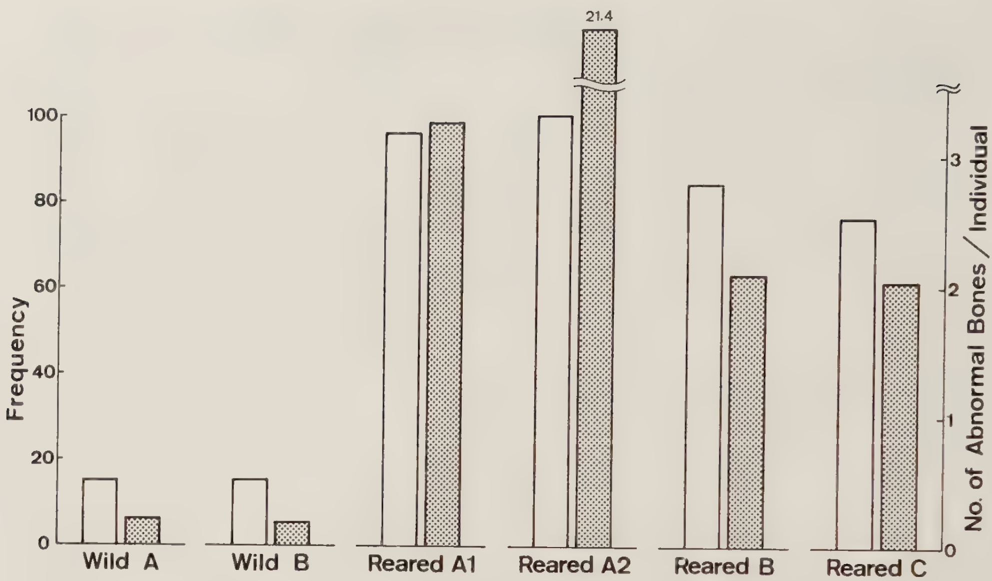


Fig. 50. Frequency (%) of specimens with at least one abnormal bone (open bar) and average number of abnormal bones per individual (stippled bar) in respective wild and reared groups.

abnormal bones in reared groups reached 96% (Reared A1), 100% (Reared A2), 84% (Reared B) and 76% (Reared C). The average number of abnormal bones was 3.28 (Reared A1), 2.10 (Reared B), 2.04 (Reared C) and an extremely high value 21.4 (Reared A2) per individual.

Arrangement of vertebra-pterygiophore

Figure 51 shows the schematic representations of the arrangement of vertebra-pterygiophore in Wild A and Reared A1, e.g., posterior two predorsals inserting between the first and second neural spines. Open area within bars indicates the percentage of specimens with normal insertion of respective predorsal and dorsal and anal pterygiophores in relation to the neural or haemal spine, and solid area indicates the percentage of specimens with abnormal insertion. The tip of the 20th proximal radial of the dorsal fin-supports did not insert into interneural space but is located slightly anteriorly or posteriorly above the tip of the 16th neural spine in both wild and reared groups. Since the location was rather indistinct, the arrangement of the 20th proximal radial was excluded from consideration, although the slight posterior location of the proximal radial tip from the 16th neural spine was treated as normal arrangement in the figure. In Wild A, a few abnormal insertions were observed in the posterior region of the dorsal pterygiophores and almost throughout the anal pterygiophores. In Reared A1, abnormal insertions were concentrated on the first, 12th, 14th, 16th and 18th proximal radials of the dorsal

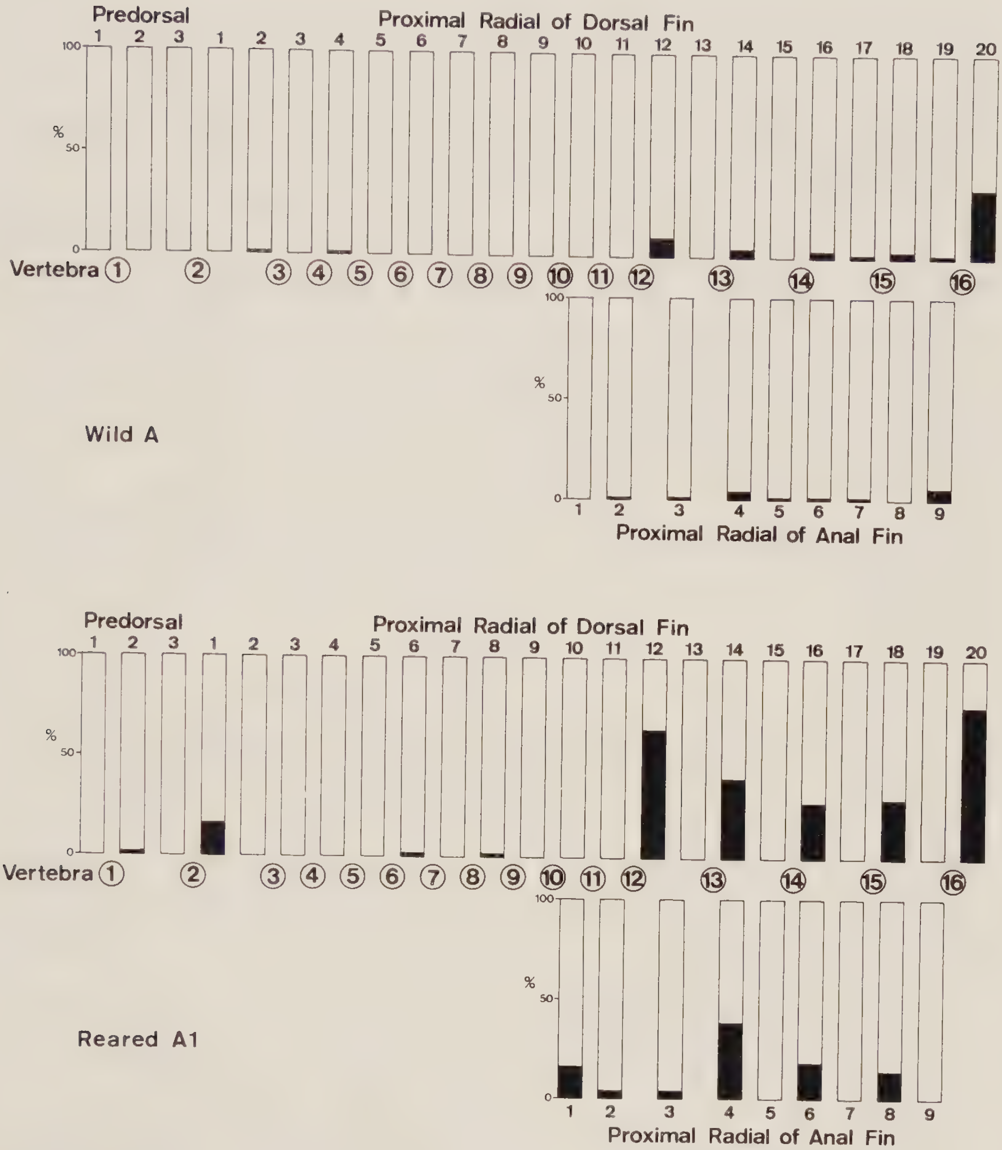


Fig. 51. Shematic representations of vertebra-pterygiophore arrangement in Wild A and Reared A1 groups. Bars indicate frequency (%) of normal (open) and abnormal (solid) insertion of predorsal or proximal radial between vertebrae.

fin-supports, and the first, fourth, sixth and eighth proximal radials of the anal fin-supports. 64% of specimens; the highest in all proximal radials except the 20th one of the dorsal fin-supports, had abnormal insertion of the 12th proximal radial of the dorsal fin-supports. In these specimens, the 12th proximal radial inserted into the anterior interneural space, i.e., between the 11th and 12th neural spines,

together with the 11th proximal radials. In many cases, abnormal insertion of the 12th proximal radial of the dorsal fin-supports was accompanied with that of the 14th, 16th and 18th proximal radials. These alternate proximal radials also inserted into anterior interneural space. Similar observations were made in the fourth, sixth and eighth proximal radials of the anal fin-supports.

The distribution and frequency of the abnormal arrangement of vertebra-pterygiophore were almost similar between Wild A and Wild B as well as between Reared A1 and Reared B. In Wild A and Wild B, the average number of abnormal arrangements per individual was 0.35 and 0.27, and specimens having at least one abnormal arrangement reached 15.0% and 18.6%, respectively. On the other hand, in Reared A1 and Reared B, the average number of abnormal arrangements per individual was 2.78 and 2.12, and specimens having at least one abnormal arrangement reached 86.0% and 76.0%, respectively.

The percentage of specimens having either an abnormal bone or abnormal arrangement of vertebra-pterygiophore was 26.0% in Wild A and 28.8% in Wild B, while reaching 100% and 94% in Reared A1 and Reared B, respectively.

Discussion

FOWLER[121)] pointed out in his review that laboratory-reared fishes are generally more variable in meristic characters than natural populations of the same species. Recently, LAU and SHAFLAND[41)] noted that reared specimens of *Centropomus undecimalis* have more meristic variations than wild specimens, although no quantitative analysis was undertaken. The present data clearly shows that reared *P. major* have considerably more variations in the numbers of the vertebrae, pleural and dorsal ribs, and fin rays than wild specimens.

In reared specimens, accurate counting of the number of the vertebrae is rather difficult because of fusion and reduction. TANIGUCHI *et al.*[132)] reported higher incidences of reared *P. major* with 23 vertebrae (maximum, 19.6%) than the present data. In this respect, their results may include fusion or reduction of the centrum. Wide variations recognized in the number of the dorsal fin rays are partly influenced by the abnormal rays such as excess small spine and intermediate ray. The fused rays are often observed in the caudal fin. Therefore, it is suggested that meristic variations in these characters of reared specimens may be to a certain extent derived by abnormal formation of the bone and ray, in addition to normal variations.

The number of the pectoral fin rays in reared specimens tends to be lower than in wild specimens, although the abnormal rays are not observed in the former. Recounting of the number of the dorsal fin rays after eliminating the influence of

the abnormal rays also shows similar tendency. Lower meristic counts in reared fishes than in wild fishes were also reported in the vertebra of *Gadus callarias*[133] and the scale of *Salmo kamloops*.[134] Recently, TATEISHI and IKEDA[135] found that reared *P. major* has lower average number of the pectoral fin rays than wild one, and TATEISHI *et al.*[136] tried to use this characteristic to identify artificially-released *P. major* from wild one. Lower counts of some meristic characters in reared fishes may be derived by environmental differences, e.g., temperature, between wild and rearing conditions as reviewed by FOWLER[121] and BARLOW.[137]

High incidences of morphological abnormalities in reared fishes have been described in many literature. For example, BARAHONA-FERNANDES[127] reported that about 90% specimens of reared *Dicentrarchus labrax* have opercular abnormality. KOMADA[138] compared wild and reared specimens of *Plecoglossus altivelis* and indicated that the frequency of external morphological abnormalities in reared specimens is about 100-500 times higher than in wild ones and the frequency of deformed vertebrae in the former is about 8-15 times higher. In *P. major* examined here, external morthological abnormalities except the shortened jaws in reared specimens are rarely observed. The majority of specimens looks like "normal" in appearance. But the detailed examination of bones on cleared and stained specimens reveals that the frequency of specimens with abnormal bones in reared groups is at least 5 times higher than in wild groups. The number of abnormal bones per individual in reared groups is at least 10 times higher. Moreover, high incidence of the abnormal arrangement of vertebra-pterygiophore is also detected in reared groups. Specimens with either abnormal bone or abnormal arrangement of vertebra-pterygiophore amount to 94% and 100% in two reared groups. It should be noted that there are numerous skeletal abnormalities even in externally "normal" reared specimens.

Recently, it was clarified that the lordosis found in *P. major* is related to swimbladder deformation mainly caused by nutritional deficiency.[139-142] The lordosis is derived by secondary deformation of the centra.[14] On the other hand, the majority of skeletal abnormalities shown here seems to be directly derived by abnormal bone formation such as fusion, reduction or over formation. The cause of such abnormal bone formation now remains to be uncertain. TANIGUCHI *et al.*[132] suggested the possibility of genetic influence on skeletal abnormalities of *P. major*. In the present study, Reared A1 and A2 were reared from eggs spawned by the same parental stock, in respective tanks. Reared A2, however, had considerably higher incidence of abnormal bones than Reared A1. Therefore, it is suggested that rearing conditions may more intensely influence abnormal bone formation than genetic factors. The highest incidence of abnormal bones among 12 types is different in the four reared groups (Fig. 49). It is assumed that different factors in respective

rearing conditions may be related to different types of abnormal bone formation

IV. GENERAL DISCUSSION

Ontogenetic Intervals

Up to the present, numerous attempts to divide ontogenetic intervals in the course of fish development have been made by many authors. WATANABE and HATTORI[143] and SNYDER[144] arranged and reviewed ontogenetic intervals presented previously. Among the ontogenetic intervals recommended by respective authors, there are some differences in definition to divide intervals and terms. In Japan, ontogenetic intervals of UCHIDA and DOTSU[145] have been generally adopted[146] which bear a resemblance to those of HUBBS[147] in some respects. In regard to the "larval period", American larval investigators mostly conform to five divisions based on the notochord flexion, which were recommended by AHLSTROM.[144]

It is an important problem to realize whether such ontogenetic intervals are only "staging" for identification, i.e., arbitrary divisions in fish ontogeny, or natural intervals precisely based on structural and functional changes. The intervals based on the notochord flexion noted above and three intervals based only on fin formation which consist of protolarval, mesolarval and metalarval ones[144] seem to be included into the former category.

On the other hand, BALON[49,148-150] emphasized the significance of developmental biology based on detailed observations of structural and functional changes in fish ontogeny. He proposed the "saltation theory" that development is not a gradual process but a saltatory process and a combination of qualitative changes in form and function creates boundaries between a succession of quantitative intervals. He also defined a hierarchical system of intervals which consists of periods, phases and steps. This theory is apparently a modification of VASNETSOV-KRYZHANOVSKY's "etap theory". According to his proposals, step is the shortest natural interval of ontogeny separated by thresholds, a combination of functional (qualitative) leaps, and period is the longest interval separated by the strong thresholds.[149,150] Phase is the second longest interval into which period is divided as a convenient means of morphophysiological identification of developmental levels[148,149] or as morphophysiological units for identification purposes.[150] These ontogenetic intervals seem to be still an incomplete or developing system, because some modifications are found in terms and defintion of intervals in his literature. For example, eleutheroembryo, protopterygiolarva and pterygiolarva of BALON[148] are respectively altered to free embryo, finfold larva and finformed larva of BALON.[150] Such alteration is presumably made to eliminate the complexity of the former terms as pointed out by RICH-

ARDS.[151] Moreover, BALON[148] recognized the alevin phase in the juvenile period of salmonids, but later the alevin phase is replaced by an independent interval of the alevin period as a larval vestige.[49,149,150] Minute differences in the definition of phase can be detected, e.g., it is of "no" saltatory significance[149] or "lesser" saltatory significance.[150] BALON[148] suggested that some organs such as the scales develop only later in the juvenile period, while BALON[49] described that the scales begin to develop and calcify at the beginning of the juvenile period. In spite of its present incomplete state, it is likely that his proposals are worth inspecting for developmental biologists, because he attempted to detect natural intervals and not artificial ones for identification.

In *P. major*, KITAJIMA[13] recognized five long intervals after hatching which consist of larva (2.3 mm TL–10 mm FL), juvenile (10–40 mm FL), young (40–90 mm FL), immature (90–230 mm FL) and adult (beyond 230 mm FL), which are similar to those of UCHIDA and DOTSU.[145] The larval interval is divided into the prelarval (2.3–3.2 mm TL) and postlarval (3.2 mm TL–10 mm FL) ones, and the latter is subdivided into the first (3.2–6 mm TL) and second (6 mm TL–10 mm FL) ones. The juvenile interval is also divided into the first (10–20 mm FL) and second (20–40 mm FL) ones. These intervals are mostly based on observations of external morphology and inflection points of external morphometric measurements. Therefore, it would necessary to examine whether these correspond to natural intervals accompanied with qualitative changes in structure and function including internal organs.

Figure 19 shows the histogram of developmental changes of all osteological elements against total length, for total understanding of the osteological development as an aggregation of qualitative changes. This histogram seems to be divided into three major intervals delimited at about 3 and 10 mm TL (less than 3 mm, form 3 to 10 mm and beyond 10 mm TL). The transformation from prelarva to postlarva is usually characterized by yolk absorption, and first-feeding begins. Before the first-feeding stage, it is necessary that elements which enable first-feeding are formed and prepared to become functional. Among 18 changes which occur between 2.5 and 3 mm TL, 14 belong to the head skeleton except the neurocranium and are mostly related to opening and closing of the mouth and expanding and narrowing of the buccal and branchial cavities. 3 changes belong to the pectoral fin-supports and are related to pectoral propulsion. These are apparently concerned with functions to catch food organisms. Furthermore, the superficial red fibers in the myotomal muscle acquire the metabolic features shortly before first-feeding stage, as shown in the present study (Chapter II-1-1). It permits initial sustained slow swimming ability for searching food organisms. It is clarified that the basic structures of the digestive system[10] and the visual system[9] are formed by the time of first-feeding in *P. major*. Therefore, it is concluded that the transformation from prelarva

to postlarva undergoes a saltatory process of many qualitative changes forming a strong threshold between periods. I prefer to consider the prelarval interval to be a phase (free embryo) in the embryonic period, following BAION,[49,148–150] because significant structural changes in embryo can be scarecely detected around hatching.

The second boundary between periods is recognized at about 10 mm TL, as a concave in the histogram of osteological developmental changes. It signifies the completion of basic osteological structures. It seems that many osteological elements are formed before the transformation from postlarva to juvenile as well as that from prelarva to postlarva. The structures of the myotomal muscle and head and fin muscles are almost completed by this transition. TANAKA[12] suggested the formation of the pyloric caecum as a key character of the transition from postlarva to juvenile in the digestive development, at 30 days old specimen (10.5 mm). Usually, the full complement of the fin ray number is used as an external key character of the transition from postlarva to juvenile. This definition, however, seems to be ambiguous as to whether it means the full complement of ray numbers in particular fins or all fins and whether it includes the differentiation to spine and segmented soft ray or not. SNYDER[144] proposed the segmentation of the principal rays in all fins as a key character from metalarva to juvenile. But, this character is not suitable for *P. major*, because the segmentation in the pectoral fin rays is completed considerably later. FUKUHARA[17] suggested a boundary at about 8 mm BL (about 9.88 mm TL) form postlarva to juvenile, based on observations of fin ray segmentation in *P. major*. However, his definition seems to be insufficient as a key character. Therefore, I want to propose the full complement of spines and segmented soft rays of the dorsal and anal fins as an external key character from postlarva to juvenile, which occurs at about 10–10.5 mm TL in reared specimens.

BALON[148] proposed two phases, protopterygiolarval (finfold larval of BALON[150]) and pterygiolarval (finformed larval of BALON[150]), in the postlarval (larval) period, as a convenient means of morphophysiological identification of developmental levels. Unfortunately, his definition about the transition between these two phases is ambiguous and frequently difficult to discern.[144] If the commencement of the differentiation of the pterygiophores and rays within the dorsal and anal fins (not caudal fin) is considered as the transitional key character between these two phases, the pterygiolarval (finformed) phase of *P. major* begins at around 6–7 mm TL. This stage almost corresponds to that of the highest peak in the osteological development shown in Fig. 19 and also of the most significant qualitative changes of the myotomal muscle, such as the differentiation of the pink fibers and stratification of the red fibers, in the postlarval (larval) period. Accordingly, the phases may be rather natural intervals with some functional changes than artificial ones. As mentioned above, KITAJIMA[13] divided the postlarval (larval) period into two intervals delimited

by about 6 mm TL. This division almost corresponds to my recognition of phases in the postlarval (larval) period. But, his definition of the second interval includes the formation of the rudiments of the caudal fin-supports and rays. My observations reveal that it occurs earlier than 6 mm TL.

BALON[148] identified no phase in the juvenile period which lasts until the beginning of first maturation, except for salmonids. On the other hand, two or three divisions within this interval, which are probably equivalent to periods respectively, were made by many authors (reviewed by WATANABE and HATTORI[143]), although their morphological definitions are indistinct. KITAJIMA[13] also recognized three divisions as period-equivalents, juvenile, young and immature, prior to the adult in *P. major*. The present study suggests that the completion of cartilaginous-osteological structures, characterized by the formation of the accessory cartilage 7 and 8 of the caudal skeleton and the ossification of the middle radials of the dorsal and anal fin-supports, may form a boundary between ontogenetic intervals, i. e., juvenile and young phases, within the juvenile period, and fishes beyond this boundary reach adult-like structures and functions except for the organs concerned with reproduction. FUKUHARA[17] suggested a boundary at about 30 mm BL (about 40 mm TL) between juvenile and young fish (their hierarchical states in ontogenetic intervals, i.e., phases or periods, are not clarified), based on the fin development in *P. major*. He thought that the formation of 11 branched rays in the pectoral fin as the completion of fin development, but my observations reveal that large specimens of this species have 12 branched rays in the pectoral fin. It is also observed that the full complement of ray branching in the pectoral fin occurs at almost same sizes as the completion of cartilaginous-osteological structures, at least in wild specimens. Consequently, I suggest that the juvenile period (from about 10 10.5 mm TL to the first maturation size) consists of the juvenile and young phases delimited about 60–70 mm TL in reared specimens. The immature interval seems to be an artificial division without structural and functional changes.

The saltatory development during the transition from one period into another, especially from prelarva (free embryo) to postlarva (larva), is generally accepted (e.g. TANAKA[152]), but the existence of steps, the shortest interval in fish ontogeny bordered by developmental thresholds,[148,149] is not sufficiently proved. Indeed, BALON[49] admitted that limited observations rarely allow for exact recognition and placement of all the existing thresholds (between steps). It is recognized that the histogram of osteological developmental changes (Fig. 19) includes several peaks (e.g. in 3.5–4.0 mm, 5.0–5.5 mm, 6.5–7.0 mm, 8.0–8.5 mm TL, etc.) within the postlarval (larval) and also juvenile periods. These peaks may suggest the thresholds between steps of osteological development. In this respect, further evidence should be accumulated, including the structural and functional changes in many other or-

gans, for exact recognition of steps as natural intervals in fish ontogeny.

Ontogenetic intervals should be defined on the basis of structural and functional changes in organogenesis. Size is only a rough standard of developmental levels, because fish reaches a certain developmental stage at various sizes. For example, reared *P. major* showed a relatively wide range and overlapping of sizes at the respective stages of fin development.[17] The mode of size in specimens reaching a certain stage may be somewhat different between the rearing experiments. The present study showed the presence of size differences in the osteological development including the fin ray development which is useful for interval recognition, between wild and reared specimens. Therefore, it should be noticed that ontogenetic intervals can not be precisely expressed at size ranges in common with wild and reared specimens.

Swimming and Feeding Functions

P. major is hatched at a much less advanced condition similar to other fishes hatched from small pelagic eggs.[10] The eyes are not pigmented and the lens and retina poorly differentiate.[9] The mouth and anus are not opened, and the rudimental digestive tract is straight and consists of undifferentiated cells.[10] Any cartilage and bone are not observed. The myotomal muscle is composed of 5–6 layers of developing white fibers containing myofibrils and a single layer of presumptive myoblasts of superficial red fibers without myofibrils. The head and fin muscles are entirely unformed. The lateral line system consists of a pair of neuromasts with cupula located at the anterior region of the otic vesicle and four pairs of placodes (rudimental neuromasts) at trunk and caudal regions.[6] Thus, newly hatched larvae (or free embryo) of *P. major* entirely lack feeding mechanism. For swimming, only the developing white fibers in the myotomal muscle are somewhat functional. It is observed that embryos within the chorion occasionally move their tails, especially just prior to hatching. Newly hatched larvae are reported to float at the water surface[10,21,153] without moving.[10,21] OKAMOTO *et al.*[6] and my observations revealed that newly hatched larvae sometimes move for a short period by beating their tails. These movements are undoubtedly carried out by developing white fibers in the myotomal muscle, although they are too short to be called "swimming".

Swimming modes in the prelarval (free embryonic) interval of *P. major* were reported by YAMASHITA.[153] According to his observations, prelarvae about 10 hours old (observation in 1961) or 6 hours old (observation in 1963) after haching begin to sink with their heads downward and subsequently exibit an upward and downward motion. TSUDA and SAKAMOTO[154] analyzed such upward and downward motion in prelarvae by means of spectral analysis with time-series data and revealed that

the motion is conducted at random in prelavae about 6 hours old after hatching but it becomes reqular and also shows diurnal rhythm (i. e. disappearance of such periodic motion at night) beyond about 24 hours after hatching. The ecological significance of this upward and downward motion in prelarvae remains to be answered. It is assumed that this motion may be related to diffusion, with the aid of ocean current in the natural environment, for individuals to obtain satisfactory food organisms at later stages. YAMASHITA[153] observed the burst rush toward various directions in about two days old larvae after hatching, in addition to the upward and downward motion. Since this rush is conspicuously caused by something approaching toward them, he suggested that the reaction in moving against external stimulus, i. e. avoidance from predators, begins to appear at this stage. YAMASHITA[7] pointed out that this stage corresponds to the completion of cupulae on neuromasts located from the head to caudal region. The pigmentation and cone formatmon in the eye, recognized in 36 hours old larvae after hatching,[9] may be also related to this type of motion. It is obvious that the upward and downward motion and the burst rush are conducted by only the myotomal muscle, since the pectoral fin muscles in a 38.5 hours old larva from hatching are still partly at the state of myoblasts without myofibrils. In about two days old larvae, the superficial cells in the myotomal muscle do not have enough metabolic properties to function as red fibers yet. Therefore, it is considered that the white fibers mostly perform the upward and downward motion and the burst rush. This seems to be the reason why both types of motion are shortly intermittent.

Histochemical characters of the superficial cells are distinguishable from the white fibers in 64.5 hours old larvae. The superficial cells seem to be able to serve as the red fibers. The pectoral fin muscles at around this stage consist of two flat muscles lying on both sides of the fin plate cartilage. These muscle fibers show histochemical staining like the red fibers in the myotomal muscle. In my observations, actual swimming and feeding activities, with larval body maintained horizontally, begin at about 10 hours later after the above stage. The appearance of body equilibrium is probably related to the development of the otic vesicles. The larvae at this stage swim by both the undulation of the caudal region and fanning of the pectoral fins for searching food organisms. Swimming lasts several seconds, followed by motionless resting for a moment. When they find food organisms, they rush on them within a short distance. Sustained slow swimming for searching food organisms at this stage is still elementary, intermittent and different from cruising at later stages. This could mean that the superficial red fibers performing this type of swimming may be still apt to become fatigued. This may be related to the low rate of successful food uptake of larvae at first-feeding stage.[155,156]

The basic structures concerning feeding function, especially in the bones and muscles of the head region, are formed shortly prior to first-feeding stage. The majority of osteological developmental changes occurring in 2.5–3.0 mm TL (shown in Fig. 19) is mostly related to this function. For example, the maxillary and Meckel's cartilage frame the upper and lower jaws, respectively, and the cartilaginous suspensorium supports the membraneous operculum. The adductor mandibulae as a compound of A1 and Aw attached to the Meckel's cartilage acts in closing the mouth, and the protractor hyoidei and sternohyoideus act in opening the mouth. Larvae at first-feeding stage attack food organisms, open the mouth, and try to swallow them by closing it. Since the above muscles are considerably slender at early stages, the opening and closing force of the mouth seems to be rather weak, even if the levator arcus palatini may serve as a swallowing pump by expanding the buccal and branchial cavities. It may also be the reason for the low rate of successful food uptake of larvae at first-feeding stage.[155,156]

It is generally accepted that success of first-feeding is most important for larval survival. In *Pleuronectes platessa* larvae attempting to feed for the first time, RILEY [157] reported that the snapping action is not repeated for some time, often for over half an hour, if a larva failed to take a nauplius after several attempts. *P. major* larvae at first-feeding stage have a small amount of yolk and an oil globule, but they are rapidly exhausted after initial attempts at feeding. Subsequent failure to feed significantly influences larval tissues. For example, starved larvae for 3.5 days after yolk absorption have only one half of the total cross-sectioned area of the white fibers in the myotomal muscle at first-feeding stage, with no increase in number of the white fibers during starved period. Reduced white fibers lose constracting function for burst rush to avoid predators and also for attacking food organisms, which the larvae are then no longer able to take, even if they exist abundantly. It is considered that similar changes probably occur in the pectoral fin and head muscles. The degeneration of these muscles would weaken the abilities of propulsion and opening-closing mechanism of the jaws. It is also indicated that early starvation causes degenerative changes in the digestive organs in *Seriola quinqueradiata*,[158] *Engraulis mordax*,[159] *Clupea harengus* and *Pleuronectes platessa*,[160] *Trachurus symmetricus*,[161] *Cyprinus carpio*,[162] *Chaenogobius annularis*[163] and so on. EHRLICH *el al.*[160] showed that the external morphometrics such as the pectoral angle and the eye height to head height ratio change during starvation in *C. harengus* and *P. platessa*. Thus, failure of feeding at early stages rapidly decreases the swimming and feeding functions of larvae, causing subsequent failure of feeding and predation and ultimately death. On the other hand, success of first-feeding causes proliferation and hypertrophy of the white and superficial red fibers in the myotomal muscle as well as further development of the head region, which then

increase the possibility for successive feeding and avoidance from predators.

After first-feeding stage, the duration of swimming period becomes longer and longer as the larvae grow, although their swimming mode is still intermittint. It enables the larvae to search food organisms over a broader area than at preceding stages. KOHNO *et al.*[15] reported that *P. major* larvae up to about 5.00 mm NL are not capable of swimming actively, but this interpretation is undoubtedly due to insufficient observations of larval behaviour, as mentioned previously. FUKUHARA and KISHIDA[21] showed that swimming activity increases remarkably in *P. major* larvae beyond 20 days after hatching (about 6 mm SL). TANAKA[164] suggested that the collecting efficiency of wild larvae by larval net considerably decreases beyond about 7 mm TL. MORI[165] also interpreted that the avoidance of wild larvae from larval net increases beyond about 6 mm TL. As to the cause of such an increase in swimming activity at about 6–7 mm TL, KOHNO *et al.*[15] and FUKUHARA[2] suggested a relationship with unpaired fin development, especially the caudal fin. Although fin development certainly increases the swimming activity by increasing rowing resistance to water, the muscle development seems to be more directly related to it. In the myotomal muscle, the differentiation of the pink fibers and stratification of the red fibers occur at around this stage. It seems that the stratification of the red fibers gradually begins to enable the larvae to perform true sustained slow swimming. The rapid increase in size and number of the white fibers improves the ability for burst swimming. The intermediate type of swimming suggested by JOHNSTON *et al.*[74] begins to be possible by the differentiation of the pink fibers. In the caudal fin muscles, all components are formed at 6 mm TL, although some of them are still very small. As most parts of these muscles consist of fibers resembling the red and pink ones, it is suggested that the caudal fin muscles are mainly functional for sustained slow and intermediate swimming by moving the caudal skeleton and rays. The increase in burst swimming activity enhances both avoidance ability from predators and attacking food organisms, and consequently the possibility to survive. The increase in sustained slow swimming and intermediate swimming activities not only gives more chances to encounter food organisms but also may contribute to "active inshore migration" as suggested by TANAKA.[164]

In regard to the feeding function of *P. major*, KOHNO *et al.*[15] suggested that the mode changes from swallowing to biting through a transitional stage (5–7 mm NL), based on the development of the jaws and pharyngeal teeth and the change of the premaxillary length-gape length ratio. On the other hand, KITAJIMA[13] pointed out that the larvae during the postlarval interval mostly obtain food organisms by swallowing because the jaw teeth are too small to function. He observed that the early juveniles with functional jaw teeth become capable of biting food organisms such as minced meat, and cannibalism begins around this stage. Cannibalism seems to be

indicative of the beginning of biting function. YAMAGUCHI[1] mentioned that the cannibalistic fish is at least beyond 8.9 mm TL. Therefore, it is considered that the biting function practically begins at a larger size than the one KOHNO *et al.*[15] suggested. The adductor mandibulae, which is a small rod-like muscle at first-feeding stage, gradually expands during the postlarval interval. It increases the force of pulling up the lower jaw and enables the larvae to capture larger and nimbler prey organisms, although the maximum food size available for the larvae is restricted by their mouth size.[166] The early juvenile with sharp canine teeth and enlarged adductor mandibulae, shown in Fig. 35, seems to be capable of biting out food organisms larger than its mouth size.

The swimming function is not only limited to forward locomotion. As the larvae grow, more complicated locomotion becomes possible probably by the development of fins. GOSLINE[167] reviewed the functions of respective fins as follows. The anterior spinous region of the dorsal fin in higher teleosts serves as a rudder as well as a keel when a fish makes a right and left turn. The posterior soft ray region of the dorsal fin is used to stabilize the forward course, to aid forward locomotion, or for braking, in conjunction with the anal fin. The caudal fin is functional for forward progression and maintaining a stationary position in the water by differential swinging of the individual rays. In addition, a teleost can force the head upward by swinging the lower caudal lobe. The paired fins, namely the pectoral and pelvic fins, serve as rudders controlling the vertical course of forward movement. In higher teleosts, the extention of the pelvic fin forces the moving fish downward in the water. When the pectoral fin is extended, the uppermost ray is swung out ahead of the lower rays. It tends to push the fish upward. The pectoral fin also has the ability to maintain, by "fanning", a stationary position in the water. Furthermore, the pectoral and pelvic fins can be functional as a braking system.

In the dorsal and anal fins of *P. major*, the proximal and distal radials and rays are successively formed beyond about 6 mm TL. The formation of the dorsal and anal fin muscles such as the erectores dorsales, depressores dorsales, erectores anales and depressores anales almost corresponds to that of the dorsal and anal fin-supports and rays. The functions of these fins by erecting or depressing the rays, as mentioned above, seem to appear practically with the degeneration of the fin fold. The functions of the caudal fin, except forward progression, are conducted by differential swinging of the individual rays or lobes. The interradialis seems to be closely related to these functions. This muscle at 6.00 mm TL is very small but became considerably expanded at 11.3 mm TL. Therefore, early juveniles, at least, may be capable of maintaining a stationary position in the water and forcing the head upward by the caudal fin. The pectoral fin muscles begin to differentiate

from two flat muscles to its respective components at 6.80 mm TL. All eight muscles and a subdivision are formed at 11.3 mm TL. The arrector ventralis and arrector dorsalis attached to the uppermost ray act by swinging it and consequently pushing the fish upward. Maintaining a stationary position in the water is performed by fanning and differential swinging of the individual rays, and braking by the extention of rays. These functions seem to be impossible to conduct until the formation of respective muscles. The pelvic fin rays begin to be formed at about 7 mm TL and their full complement is accomplished by about 8.5 mm TL. Although the developmental process of the pelvic fin muscles was not investigated in this study, the functions of the pelvic fin, as a rudder controlling the downward course of forward movement and as a brake, presumably become possible beyond the full complement of rays. Thus, the development of fins including fin-supports and supporting muscles are mostly related to the maneuverability of fish locomotion. It seems that the maneuverability begins to appear in late postlarvae and sufficiently functions in early juveniles. The development of maneuverability enables fishes to pursue and capture their prey organisms which can make more complicated movement and also increases the possiblity of successful avoidance from predators. Therefore, it is thought that the development of maneuverability considerably enhances the possibility of fish survival as well as increasing forward swimming ability.

Problems in Seedling of the Red Sea Bream

One of the most important problems in seedling of *P. major* is the production of healthy and vigorous fish which can successfully survive and grow in the natural environment after release. Unfortunately; it is generally accepted that reared fish is weaker and less resistant in some respects than wild one.

Blaxter[168] mentioned that hatchery-reared flatfish does not survive well when transferred to the sea, presumably because of high predation resulting from poor swimming performance. The unusual environment in rearing condition may lead to impaired locomotor performance,[113] for example, high density of food organisms, slow water flow and narrow space in tanks probably decrease locomotor performance of larvae. Impaired locomotor performance may prevent the development of muscular systems[113] and result in poor swimming ability. Greer-Walker[169] indicated that exercise considerably increases the diameter of the red and white fibers in the myotomal muscle of the coalfish, *Gadus virens*. Therefore, it is suggested that special exercise before release may be needed to increase swimming ability as well as rearing in relatively low density of food organisms, appropriate water flow, etc. Blaxter[113] also pointed out that reared fish has low ash contents

as a result of poor ossification of the skeleton. Poor ossification of the skeleton and fin rays decreases the rowing resistance to water and consequently diminishes swimming ability. This problem seems to require further nutritional improvement of food organisms. Moreover, severe bone abnormality with body deformation such as lordosis certainly decreases swimming ability. Slight bone abnormality almost without body deformation, shown in the present study, may also influence it. For example, fusion of the centra seems to diminish flexibility in bending the fish body and fusion of the hypurals may prevent differential swinging of the individual rays or lobes. MUNEKIYO *et al.*[170] reported high incidences of distorted or shortened pectoral fin rays in reared *P. major*. Fin-biting is often observed in rearing condition.[113] Maneuverability in swimming may be obstructed by such deformed fins. A poor swimming ability may cause low survival in the natural environment not only because of predation but also because of failure in food uptake. High incidences of jaw abnormality in reared *P. major*, shown in the present study, probably are a hindrance to the capture of natural nimble prey organisms.

Generally, it is considered that reared larvae are under "over-protected" condition, because of the high density of food organisms and lack of predators. It is also thought that high density of food organisms in rearing condition leads to faster growth than in natural condition. TANAKA,[171] however, suggested that the integrated water temperature in which wild *P. major* larvae grow to 10 mm TL is considerably low than that in reared larvae. It is indicative of faster growth of wild larvae than reared ones, in spite of the lower temperature and lower density of food organisms in natural condition. He proposed two possibilities for the relatively faster growth of wild larvae. One is due to high selection pressure in natural condition in which only faster growing larvae can survive. The other is due to inferior condition in rearing tanks for larval growth, for example, lack of variety of food organisms, high density of individuals, narrow space, pollution of rearing water, etc. Although selection pressure would be certainly high in natural condition, it seems to be doubtful whether selection pressure alone induces the difference in growth between wild and reared larvae. In this respect, the influence of inferior condition in rearing tanks to larval growth should be more sufficiently taken into consideration. TANAKA[172] reported that slowly growing larvae of *Plecoglossus altivelis* are at a more advanced developmental level of digestive organs than fast growing ones of the same size. It is suggested that size differences at the same developmental levels of osteological characters between wild and reared *P. major*, shown in the present study, may be another indication of relatively slower growth of reared larvae than wild ones, in addition to the influence of temperature differences between natural and reared conditions. In rearing condition, crowding induces stress[113] and may decrease growth rate. Crowding in rearing tanks, how-

ever, will be inevitable to some extent for intensive production of fish seedling. THEILACKER[173)] showed that *Trachurus symmetricus* larvae reared in large containers grow faster than larvae reared in small containers, at the same density of individuals and at the same food density. Therefore, to use large tanks for rearing may be one method to realize faster growth, without alleviation of crowding. Stabilization of quality of rearing water and further nutritional improvement of food organisms will be also required to increase the growth of reared larvae.

The qualitative evaluation of reared juveniles is important to estimate the effect of releasing for stocking recruits. Recently, FUKUHARA[174)] reviewed this problem. Tolerance to starvation, salinity, temperature, water flow, exposure to air and so on are suggested as methods to judge the ability to survive, but a practical useful method is not established yet. Morphological check seems to be an indirect method for this purpose. Incidence of lordosis, branchiospondylie, pugheadness, operculum abnormality, abnormal pigmentation and so on which can be detected by external observations is a simple index for qualitative evaluation. Incidence of slight bone abnormality and abnormal arrangement of vertebra-pterygiophore which are almost without external body deformation seems to be a more detailed index, although clearing and staining method is needed. On the other hand, there is a preconceived notion that various abnormalities occur in natural condition as well as in rearing condition but they are kept at a low rate by natural selection in the former. If it is true, intensive production of healthy and vigorous larvae seems to be impossible. However, considering the facts that lordosis is a result of nutritional deficiency (e.g. WATANABE[141)]), and that incidence of albinism in reared *Paralichthys olivaceus* is conspicuously decreased by feeding of wild zooplankton,[175)] it is likely that various defects in rearing condition induce various abnormalities. Therefore, to decrease morphological abnormalities of reared larvae by improvement of rearing condition, even if they are so slight that they are apt to be overlooked, seems to be an important step to produce larvae which are similar in behavioural, physiological and biochemical aspects to wild ones. The hope of aquaculturists to produce larvae almost completely identical to wild ones in all aspects[176)] will be certainly accomplished in the future through such a step.

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マダイの骨組織と骨格筋の発達

松 岡 正 信

抄　　録

本論文では，マダイの骨組織と骨格筋の発達過程の概要を示し，仔稚魚の摂餌・遊泳機能，発育段階等について検討する。また，天然魚と人工採苗魚の形態を比較し，その差異についても言及する。

I．骨組織

二重染色透明標本や組織切片により，すべての骨組織の出現から完成に至る過程を明らかにした。その結果から，軟骨の出現，軟骨の定数化，骨化開始，骨化の完成等を一つの変化の単位として計数した。

ある魚体長において，すでに起こった変化の累積百分率は骨格系の発達程度の指標となる。そこで，4つの骨格系グループに分け，それぞれの発達程度の推移をみた。

頭蓋骨以外の頭部骨格のこの値は摂餌開始期には既に20% 近くに達し，全長10㎜では約95%に達している。このグループは呼吸や摂餌に直接関与し，仔魚の生残にとって最も基礎的な機能を果たす。脳を保護する頭蓋骨の形成開始はやや遅れるが，全長10㎜には100%に達する。脊柱や鰭支持骨・鰭条の場合は全長10㎜でも80%程度にしか達せず，体側筋を中心とする推進機能を二次的に補助あるいは制御しながら，比較的ゆるやかな発達を遂げるのであろう。

骨格系における全ての変化を計数し全長に対するヒストグラムを作成すると，いくつかのピークが認められ，これらは骨格系における発育の段階性を示唆するものと思われる。

II．体側筋

体側筋は骨格筋の中で最も大きく，推進力を生ずる主要な筋肉である。マダイの体側筋は主に赤色筋，中間筋，白色筋から成り，それぞれ，持続的，中間的，突発的運動の際に機能する。

孵化直後の仔魚では，筋原繊維を含んだ白色筋繊維が既に5～6層形成されているが，赤色筋繊維は表層に一列に並ぶ予定筋原細胞の状態で，まだ筋原繊維を含んでいない。赤色筋繊維の筋原繊維は孵化後21時間の仔魚で認められるが，赤色筋を特徴づける酵素反応や脂肪の蓄積は摂餌開始の少し前にようやく顕著になる。従って，前期仔魚に認められる断続的な運動は主に白色筋の収縮によると考えられる。赤色筋の収縮エネルギー源としての脂肪蓄積は油球の吸収期と一致し，両者の関係がうかがえる。

摂餌開始期の仔魚は一層の赤色筋の機能分化により初歩的な持続運動が可能となるが，まだ断続的な面を残している。

全長7㎜前後になると水平隔膜付近で中間筋繊維が形成され，赤色筋繊維は多層化し始める。全長11㎜の体側筋の基本構造はほぼ完成している。これらにより，次第に本来の持続運動が発現し，緩急自在な円滑な遊泳が可能になると思われる。

3筋繊維型の量的変化の一例として，切片の一断面における筋繊維数，平均筋繊維面積，総筋繊維面積を全長に対して両対数グラフにプロットすると，回帰直線の変曲点は摂餌開始期前後と6～10㎜の稚魚への移行期にほぼ集中しており，これらの時期において量的増加様式も変化することがうかがえる。また，全長10㎜の個体は摂餌開始期のものに比べ，白色筋繊維数で約30倍，総白色筋繊維面積では約90倍にも及び，この間の筋収縮力の増加は著しいと思われる。

III．頭部・鰭筋肉

頭部や鰭筋肉の発達は，連続切片から再構成して調べた。

口の開閉に関与する Protractor hyoidei や Adductor mandibulae は摂餌開始の少し前に形成されるが，非常に細く，この頃の口の開閉力は弱いものと思われる。しかし，口腔を拡げる Levator arcus palatini はかなり大きく，餌を吸い込む際に機能していると思われる。胸鰭筋肉もこの頃に軟骨板を挟んで2層形成され，体側筋の推進力を補助している。

頭部及び胸鰭筋肉は全長6～7㎜頃に大きく変化する。Protractor hyoidei は太くなり，Adductor mandibulae は3つの筋肉に分かれ，それぞれ大きくなり，口の開閉力は増大する。Adductor mandibulae 1 からは tendon が伸び，Maxillary に付着する。これにより上顎の伸出が可能となり，餌を啄む機能が発現する。胸鰭筋肉はいくつかの筋肉に分かれ始める。背鰭，臀鰭，腹鰭，尾鰭の筋肉は担鰭骨と平行して形成される。

全長11㎜までには全ての筋肉が形成され，上下左右の方向転換やブレーキなどより複雑な動きが可能となる。

以上の結果から，マダイの発育段階を区分した。これは BALON の発育段階説を基礎に置いたものである。従来の海産魚類の発育段階は識別のための便宜的な区分けに偏っている傾向があり，指標形質だけでなく実際の発育の段階性に基づいて，質的な変化の集積を量的に捉える必要があろう。

Ⅳ. 天然魚と人工採苗魚

発育は本来個体レベルの出来事であって，当然個体による変異は存在する。ある飼育群の平均的な発育経過も，別の飼育群ではやや異なるであろう。更に，成育環境の大きく異なる天然魚と人工採苗魚では発育経過や形態に違いのある可能性がある。

椎体及び背鰭，臀鰭，尾鰭，胸鰭，腹鰭の鰭条の形成過程を調べると，全長15㎜以下ではいずれにおいても人工採苗魚の方が1～2㎜小さいサイズで形成が進んでいた。逆に，骨格系の完成する時期は人工採苗魚の方が10～20㎜大きなサイズであった。

次に，稚魚の骨格を調べたところ，天然魚でも約15%の個体が異常骨を持っていたが，人工採苗魚では80～100%に達していた。これに背鰭，臀鰭の担鰭骨と脊椎骨の対応不全を加えると94～100%に達した。骨格異常の集中する箇所は飼育群によって様々であった。人工採苗魚の鰭条数変異は特に背鰭で著しく，胸鰭とともに天然魚よりやや数が少ない傾向にあった。

このように，人工採苗魚は外見からは判断できないような多くの小さな骨格異常や変異を抱えており，骨格形成と魚体サイズの関係においても天然魚とは異なっている。このような現象は他の器官系においても起こっている可能性が強い。今後，より天然魚に近い人工種苗を作るために多方面からの検討が必要であろう。

Oxygen Consumption and Biological Productivity of Mysid Population in a Small Inlet of Kyushu

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Oxygen consumption of mysid shrimps (body weight of 0.07–0.7 mg in dry weight) from Shijiki Bay, western Kyushu, Japan was measured by Winkler method at 20°C. It ranged from 2 to 10 μl O_2 per mg body dry weight per hour.

From these results, daily weight specific production and food requirement were estimated under assumptions that assimilation efficiency of food is 0.8, net production efficiency, K_2, is 0.4 and soluble organic excretion is negligible. An individual of 0.6 mg body dry weight achieved production by 6–10% of the weight a day and the 0.07 mg did by 16%. The former mysid required forage of 30–50% of body weight a day and the latter 80%.

INTRODUCTION

Mysid, a shrimp like peracarid crustacean, is a major constituent among prey animals for larval stages of red sea bream[1,2] and flounder[3,4] in the neritic waters of Japan. To estimate carrying capacity of the fish in a certain sea area biological productivity of the prey animal is to be investigated.

Physiological method is one of the practical ways to estimate the biological productivity of zooplankton.[5] When the respiratory rate is given, the productivity is obtained by using certain sound values of assimilation efficiency of food and net production efficiency K_2 (produced/assimilated) previously reported.

The present paper determined respiratory rate of the neritic mysids in an area of the temperate waters and growth rate and food requirement of the mysids were calculated from the results.

MATERIAL AND METHODS

The mysids were collected with a hand operated small plankton net (mouth frame 10 cm in height, 30 cm in width, 60 cm in side length, 0.05 mm mesh openings) by SCUBA diving in Shijiki Bay (approximate area 10 km²), Hirado Island,

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Kyushu from late June to early July 1985. The site of sampling is in the innermost part of the Bay (33°11.05′N ; 129°22.47′E) with depth of 5–7 m and with seabed of eel grass, *Zostera marina*. The eelgrass does not always grow thick in the bed. The mysid crowded and crept on the coarse sand bottom of a rift of the *Zostera* bed. Many individuals avoided the mouth of the plankton net during the tow. A batch of mysids in a tow contained not only a single species but some other species. This is probably due to continuous towing from one school to another done to keep enough specimens for the determination of the oxygen consumption. Temperature of bottom water ranged from 22.0 to 22.5°C. The samplings were made for about 30 minutes after 8 o'clock in the morning every day, and the samples were later transported to the laboratory of mother ship, RV *Yoko Maru* in which the experiments were carried out. They were contained in the water tank for 3 hours keeping water temperature at 20°C to acclimatize to the laboratory conditions.

Table 1. Species composition and body size of mysids collected by SCUBA in Shijiki Bay, June 29–July 3, 1985

Experiment no(s).	1	2	3	4–7	8,9
Species composition (percentage in the number of individuals)					
Mysidopsis surugae					
juvenile				3.4	
female			100	8.6	
male	33.3			8.6	
Proneomysis ornata					
female	33.3			31.0	
male		8.3		17.2	
Proneomysis sandoi					
juvenile		8.3		5.2	
female		50.0		12.1	
male		25.0		6.9	
Lycomysis bispina					
female		8.3		3.5	
male	33.3			3.5	
Anisomysis pelewensis					
male					63.2
juvenile					36.8
Length of body* (mm)					
Number of specimens	3	11	4	50	15
Mean	4.25	3.80	5.01	4.39	1.93
Standard deviation	0.35	0.55	0.18	0.59	0.38
Range	3.9–4.5	3.3–4.8	4.8–5.1	3.5–5.3	1.2–2.5

* Distance from the frontal edge of the carapace to the end of 6th abdominal segment

An assembly of 10–20 individuals (70–80 for smaller organisms) of mysid (s) with similar body size which were sorted from the plankton samples was put in 300 ml BOD bottle with glass fiber filtered and well aerated sea water collected from the surface of the mysid sampling site. The bottle was incubated in the 20°C container after several washing with the same water and after wrapping with aluminum foil and black plastic cloth. Antibiotics were not used. Oxygen content was determined by Winkler method after 3 hours of incubation. Sodium thiosulphate solution of a factor of 0.005 N was prepared. The oxygen content of the water by the end of the incubation amounted to 4.6–4.8 ml O_2/l, and 85–89% saturation. The batch of the animals was washed with ammonium formate to remove salts, and stored at 15°C below zero during 5 days cruise. The dry weight measurement was made after 24 hours drying at 65°C. The animals other than those used for the respiration experiment were reserved in formalin sea water, and taxonomic identification was made for each tow (Table 1).

OXYGEN CONSUMPTION

The mysids of 0.07–0.7 mg in dry weight took in 2–10 μl O_2 per 1 mg dry weight per hour (Table 2). The respiratory rate was higher in a small mysid and lower in the big one. Exp. 1 showed an extraordinary high value for the body weight. The above results well settle within the range of the previous data on the respiration of mysids at the high temperature which are compiled in Table 3. Relationship between body weight, W, and respiration, R, is generally given by a formula,

$$R = a W^b \quad \cdots\cdots (1)$$

where a and b are the constant. The constant a depends on the external environmental

Table 2. Summary of experiments of respiratory rate for mysid population collected in Shijiki Bay, June–July, 1985

Experiment no.	Date	Number of animals	Mean body dry weight (mg)	μl O_2/animal /hr	μl O_2/mg dry weight/hr
1	June 29	10	0.232	2.33	10.06
2	June 30	21	0.500	1.74	3.48
3		9	0.607	1.11	1.83
4	July 1	23	0.704	2.11	3.00
5		17	0.607	1.89	3.12
6		17	0.686	2.18	3.18
7		23	0.575	1.75	3.04
8	July 3	78	0.0708	0.382	5.39
9		68	0.0721	0.361	5.01

Table 3. Respiratory rates of mysids under the temperature higher than 10℃ reported by previous authors

Species and forms	Experimental temperature (℃)	Dry weight (mg) per animal	Respiration (μl O_2/mg dry wt./hr)	Geographical region	Reference
Siriella thompsonii	27.7 27.8	1.31-1.62	4.18-5.83	Indian Ocean	IKEDA[6]
Archaeomysis grebnitzkii	10	0.2-1.00	1.32-2.30	San Juan Is.	JAWED[7]
	10	1.20-1.97	1.09-1.39	San Juan Is.	JAWED[7]
Gastrosaccus simulans	27.5	0.45-0.66[a]	7.7-12.0	?	GAULD & RAJU[8,b]
Hypererythrops sp.	17.0	0.542	1.61	Hokkaido	IKEDA[10]
Metamysidopsis elongata					
juvenile	13.8	0.03	7.71	La Jolla	CLUTTER & THEILACKER[11]
male	13.8	0.31	3.60	La Jolla	CLUTTER & THEILACKER[11]
brooding female	13.8	0.565	2.94	La Jolla	CLUTTER & THEILACKER[11]
Hemimysis lamornae	20	1.38-2.56[c]	18.0[d]	?	GRAINGER[13]
	10	1.38-2.56[c]	9.0[d]	?	GRAINGER[13]
Neomysis awatschensis	10	0.48-1.59	1.98-3.39	San Juan Is.	JAWED[7]
	10	2.02-4.37	1.44-1.78	San Juan Is.	JAWED[7]
Neomysis integer	10	11.2[c]	1.17	Southampton	RAYMONT *et al*[14]
Acanthomysis pseudomacropsis	14.5-15.3	2.40-3.35	1.27-1.85	Hokkaido	IKEDA[6]
Acanthomysis sp.	8.9-11.0	1.89	1.64	Hokkaido	IKEDA[6]

[a] Read from the text figure by MAUCHLINE[9] (p. 148)
[b] Cited from MAUCHLINE[9] (p. 146)
[c] Calculated by a formula: W (dry wt., mg) = 0.00436L (body length, mm)$^{2.77}$ (CLUTTER & THEILACKER[11]). Body length from MAUCHLINE & MURANO[12]
[d] Wet weight equivalent to 5 times dry weight

Table 4. Previous values of b, the exponential value of body weight (W) to estimate the respiration rate (R), $R = aW^b$, in mysids and zooplankton

Species	Body weight (mg)	Temp. (°C)	b value	Reference
Mysis relicta	0.08-4.0	0-8	0.72-0.78	LASENBY & LANGFORD[17]
Mysis relicta	0.18-10.8	2-13	0.70-0.82	LASENBY & LANGFORD[17]
Archaeomysis grebnitzkii	0.20-1.97	10	0.70	JAWED[7]
Metamysidopsis elongata	0.03-0.66	13.8-18.1	0.67	CLUTTER & THEILACKER[11]
Neomysis awatschensis	0.48-4.37	10	0.62	JAWED[7]
Neomysis mirabilis			0.82	SHUSHKINA[18*]
Zooplankton	0.0003-120	10	0.78	IKEDA[6]
Zooplankton	0.0003-120	20	0.67	IKEDA[6]

* Cited from MAUCHLINE[9] (p. 149)

condition and b represents the intensity of internal metabolic factors.[15] The value of b is a function of temperature for zooplankton in general.[6] According to JAWED,[7] on the contrary, the value is different between the two species of mysid under the same experimental condition. The low value of weight specific respiration in Exp. 3 (*Mysidopsis surugae*, Table 2) may be specific to the species. The previous values of b ranged from 0.6 to 0.8 (Table 4). The relationship between R and W in the present results was given when an extraordinary value (Exp. 1) was omitted from the calculation.

Thus

$$R = 2.52\ W^{0.725} \quad \cdots\cdots (2)$$

The exponential value seems to be reasonable being the median of values obtained by the previous works. The correlation coefficient between them, 0.819, was significant ($p<0.01$).

GROWTH AND FOOD REQUIREMENT ESTIMATION

Growth and food requirement were estimated from the result of respiration measurement for 3 categories as follows; 2 monospecific (probably) populations (*Mysidopsis surugae* in Exp. 3, *Anisomysis pelewensis* in Exps. 8, 9) and a multispecies population (Exps. 2, 4-7) (Table 2).

The terms of food requirement (F) and production (P) including growth, reproduction and moulting can be calculated from the knowledge of assimilation efficiency of food (A) and the gross growth efficiency (K_1) assuming that soluble organic excretion is negligible as follows[16] :

$$A = (P + R) / F \quad \cdots\cdots (3)$$
$$K_1 = P / F \quad \cdots\cdots (4)$$

Therefore,

$$F = R / (A - K_1) \quad \cdots\cdots (5)$$
$$P = K_1 R / (A - K_1) \quad \cdots\cdots (6)$$

CLUTTER and THEILACKER[11] experimenting on Mysidae obtained an assimilation efficiency of 0.9 by carbon 14 method. LASENBY and LANGFORD[17] got 0.85 and 0.82 on *Mysis relicta* fed on *Daphnia* and chironomid larvae, respectively. These values seem higher than those of many herbivorous copepods compiled by IKEDA and MOTODA.[16] Mysids have a great variety of organic materials in their stomachs (MAUCHLINE,[9] pp. 95–97). Detritus and omnivorous feeding may bring the higher efficiency than feeding on phytoplankton with undigestable cell wall. We chose a value of 0.8 for our mysid assimilation efficiency.

The ratio of assimilated food to converted body tissue, K_2, was 0.57 in *Neomysis mirabilis*[19] (cited from MAUCHLINE,[9] p. 96) and 0.43 in *Metamysidopsis elongata*.[11] It changed with age showing higher values in early juvenile and lower ones in the adult for *Neomysis mirabilis*[20] (cited from MAUCHLINE[9], p. 97). We adopt here a value of 0.5 for K_2, and thus the gross production efficiency, K_1 is induced as 0.4 from the assimilation efficiency of 0.8 mentioned above. Substituting these into Equations (5) and (6) we get food requirement and production for mysid as

$$F = R / (0.8 - 0.4) = 2.5\ R \quad \cdots\cdots (7)$$
$$P = 0.4\ R / (0.8 - 0.4) = 1\ R \quad \cdots\cdots (8)$$

To convert oxygen data to carbon unit respiratory quotient (RQ), the molar ratio of carbon dioxide produced to oxygen consumed, is required. The RQ value for protein, 0.97, should be more appropriate for marine zooplankters (OMORI and IKEDA,[5] pp. 191–193). When the RQ is 0.97, 1 μl of oxygen combusts 0.52 μg of organic carbon ($0.97 \times 12\ \mu g$ C/22.4 μl $O_2 \times 1\ \mu l$ O_2). The carbon unit can be converted to a dry weight equivalent by a carbon/ dry weight ratio of mysid and its food material. MAUCHLINE[9] (p. 101) compiled the various data on content of carbon in dry weight in mysids. These ranged from 0.22 to 0.65 with a mean of 0.44 (Number: 11, standard deviation: 0.095). Four determinations on the mixed population of phytoplankton and copepods by CURL[21] give a mean carbon/dry weight ratio of 0.19. We adopt here the value of carbon/dry weight ratios of 0.4 and 0.2 for mysid and its prey organisms, respectively. Table 5 shows the result of

Table 5. Respiration and calculated production and food requirement in 3 categories of Shijiki Bay mysid at 20°C

Exp. no(s).	Species	Mean body dry weight (μg)	Respiration (μl O_2/animal/hr)	Respiration (μg C/animal/day)
2, 4-7	Mixed	614	1.93	24.1
3	*Mysidopsis surugae*	607	1.11	13.9
8, 9	*Anisomysis pelewensis*	71.5	0.372	4.6

Exp. no(s).	Production (μg dry wt./animal/day)	Food requirement (μg dry wt./animal/day)	Daily wt. specific production (%)	Daily wt. specific food requirement (%)
2, 4-7	60.2	301.1	9.8	49.0
3	34.6	173.2	5.7	28.5
8,9	11.6	58.0	16.2	81.1

calculation of respiration, production and food requirement from the Equations (7) and (8) at a temperature of 20°C in three categories of mysid in Shijiki Bay. They achieved production of 6-10% of their body weight per day in the population with body dry weight of 600 μg and 16% in the 70 μg. They are set in the range of values, 1-13%, enumerated by MAUCHLINE[9] (p. 219) refering to the previous works on *Paramysis intermedia, Mysis relicta, Schistomysis spiritus* and *Neomysis mirabilis*. An approximate food requirement ranged from 30 to 50% of body weight a day in 600μg animals, and 80% in the 70μg. These values may somewhat be lowered if they depend for food not on phytoplankton but on animals.

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九州平戸島志々伎湾におけるアミ類群集の酸素消費と生物生産

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抄　　録

1985年6月下旬から7月上旬にかけて長崎県平戸島南部の志々伎湾野子地先，海深5～7 mのアマモ場内の砂底においてスキューバ潜水により手持ちの小型プランクトンネット（口枠の高さ10cm，幅30cm，側長60cm，網目幅0.05mm）を用いてアミ類を採集し，調査船陽光丸の実験室でアミ類の酸素消費量を測定した．アミ類は敏捷でダイバーが操るネットの口から逃避するものが多かったので，測定に充分な個体数を確保するためには一網で数個のスクールについて捕集することになった．一網あたりふつう10～20個体，体が小型のものについては70～80個体のアミを容量300m*l*の酸素瓶に入れ，対照とあわせて20℃に保たれた水槽に収容して3時間後にウィンクラー法によって酸素量を測定した．以上の手順はウォーター・ボトル法の常法にしたがった．実験に供した個体は乾燥して重量を測り，残りの個体はホルマリン固定して種を同定した．

供試アミの平均体重（乾燥重量）は0.07～0.7mgの範囲にあり，体重1mgあたりの酸素消費量は1時間に2ないし10 μl O_2 であった．溶存有機物の排せつが無視できるものとし，既往の研究から食物消化効率を0.8，純成長効率（K_2）を0.4，呼吸商を0.97，アミ自体の炭素/乾重量比を0.4，アミの餌料のそれを0.2と仮定して生産速度と餌料要求量を算出した．体重（乾重量）0.6mgのアミは1日に体重の30～50%の餌料を要して6～10%の成長，成熟，脱皮をこみにした生産をあげ，そして0.07mgのものは1日に体重の80%を食して16%ずつ生産することになる．

CONTENTS

SEIKAI REGIONAL FISHERIES RESEARCH LABORATORY
49 KOKUBU-MACHI, NAGASAKI 850
JAPAN